FACTORS AFFECTING THE COMPOSITION OF BODY FLUIDS IN MATERNAL, FETAL AND NEONATAL GOATS AND SHEEP

by

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TO DAD AND MUM

DECLARATION

This thesis was composed by the undersigned who was also responsible for the research work described herein. The project was carried out under the supervision of Dr.D.J.Mellor and Dr.E.Hobson at the Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh.

ABSTRACT

Factors affecting the composition of plasma, amniotic fluid, allantoic fluid, fetal urine and fetal ruminal and abomasal fluid were studied.

Effects of laboratory conditions and experimental procedures on plasma composition were examined in goats and sheep. It took animals 1-2 weeks to adapt to the laboratory and 3-6 weeks of handling to adjust to prolonged procedures such as repeated venepuncture.

Surgical insertion of uterine catheters was associated with changes in feed intake and plasma composition which were present after operation for up to seven days in sheep and 12 days in goats.

Gestational changes in fetal fluid composition were examined in goats. Amniotic fluid composition was similar to that in sheep but differences in allantoic fluid composition were observed. It was not possible to say whether these differences were related to species differences in hormone production during pregnancy because exogenous progesterone was given in an attempt to reduce the high incidence of postoperative abortion encountered in goats.

Gestational changes in the composition of ruminal and abomasal fluid were studied in sheep. Values for sodium and potassium concentrations and osmolality of the amniotic, ruminal and abomasal

fluids suggested that amniotic fluid was modified during its passage between the amniotic sac and the rumen.

Effects of fetal adrenocorticotrophin and corticosterone infusion on the composition of allantoic fluid and fetal urine were studied in sheep. Results were consistent with an hypothesis that fetal corticosteroids act on ion pumps in the chorioallantois and fetal kidneys to alter the sodium and potassium concentrations of allantoic fluid and fetal urine.

Changes in blood composition during the first 12 hours of life were observed in lambs and kids using a non-surgical method of catheterising the aortae and venae cava. Marked changes in composition occurred as breathing was established and after sucking. Effects of hypothermia and haemorrhage on blood composition were also reported.

Finally, the 'chronic' approach to fetal physiology was critically evaluated. In this work the term 'chronic' has been used to describe the continuous study of conscious, catheterised animals.

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LIST OF CONTENTS

CHAPTER ONE

INTRODUCTION

The relative inaccessibility of the conceptus within the uterus of the pregnant animal is one of the greatest problems facing the fetal physiologist. In early studies, the ruminant conceptus was examined in large numbers of animals killed at known gestational ages. Although adequate for anatomical studies this approach could give little information about the continuous physiological processes taking place in the developing fetus. This difficulty was partly overcome by the use of anaesthetics which allowed a variety of experiments to be carried out on the uterus and its contents in live, anaesthetised animals. Experiments of this nature were necessarily of short duration and are now commonly called '<u>acute</u>' experiments.

In retrospect, we regard the considerable manipulations of the uterus, placenta or fetus in many acute experiments as unphysiological. Until the late 1960s, relatively extreme procedures such as partial or complete exteriorisation of the fetus (e.g. Alexander, Nixon, Widdas and Wohlzogen, 1958a; Comline and Silver, 1961) or perfusion of the isolated fetus or placenta (e.g. Alexander, Huggett, Nixon and Widdas, 1955; Alexander, Britton and Nixon, 1966, 1970a; Britton, Huggett and Nixon, 1967) were regarded as acceptable methods of obtaining information about the conceptus. This view changed only when the implications of the first report on the composition of fetal blood in conscious animals (Meschia, Cotter, Breachnach and Barron, 1965) were realised. The progressive fetal hypoxia observed in acute experiments as pregnancy advanced was not found in these

conscious animals. This implied and confirmed that acute procedures severely stress the fetus.

Meschia et al. (1965) obtained blood from fetuses in conscious goats and sheep for periods of up to 29 days after surgically inserting catheters into the umbilical vessels. This new 'chronic' approach extended the period of observation in individual fetuses from hours to days or weeks as well as drawing attention to the stress effects of acute experiments. This approach is now widely used in the study of fetal physiology. In addition to modifications of Meschia's method of umbilical catheterisation (Comline and Silver, 1970; Young, Creasy and Rudolph, 1974; Mellor and Matheson, 1975), it is now possible to catheterise the fetal aorta and vena cava (Comline and Silver, 1972; Mellor and Matheson, 1975) a carotid artery and jugular vein (e.g. Bassett and Thorburn, 1969; Joelsson, Barton, Daniel, James and Adamsons, 1970) the peritoneal cavity (Liggins, 1966), urethra(Buddingh, Parker, Ishizaki and Tyler, 1971), bladder (Gresham, Rankin, Makowski, Meschia and Battaglia, 1972; Mellor, Williams and Matheson, 1972), trachea (Dawes, Fox, Leduc, Liggins and Richards, 1972; Adamson, Brodecky, Lambert, Maloney, Richie and Walker, 1973), rumen and abomasum of the fetus (Pearson and Mellor, 1976) and the amniotic and allantoic sacs (Mellor, 1970a). The application of electronic monitoring and recording equipment has allowed fetal breathing, electroencephalogram, electrocardiogram, heart rate, arterial pressure and body temperature (Dawes et al., 1972; Joelsson et al., 1974) and fetal swallowing (Bradley and Mistretta, 1973) to be observed in utero. Intrauterine pressure changes during pregnancy and particularly during labour (Hindson,

Schofield, Turner and Wolff, 1965) can also be recorded.

The advantages of the chronic approach have been justifiably emphasised, but until recently (Mellor and Slater, 1973a; Shelley, 1973; Comline and Silver, 1974) the limitations have received little attention. Application of the above techniques has shown that the fetus is extremely sensitive to changes in the mother. In sheep, for example, changes in the ambient temperature, level of nutrition, feeding pattern or water balance of the mother are associated with changes in the composition of fetal urine and allantoic fluid (Mellor and Slater, 1971, 1973b c; Mellor, Slater and Matheson, 1975a), and disturbing the mother by pen cleaning can cause an increase in the rate of fetal breathing (Dawes, 1973). This degree of fetal sensitivity introduces problems of experimental design, not the least of which is the need to assess or regulate effects of environment (including the presence of the experimenter) and surgery on the animal.

In 1972 the author joined a group actively engaged in fetal sheep research. Because of the extensive use of the chronically catheterised ruminant, it was felt that a critical appraisal of some associated procedures would be worthwhile. One of the main objectives of the work has been to assess laboratory studies of pregnancy, beginning in early gestation when the animals were brought indoors and ending when the mother and newborn were returned to a covered yard 2-3 days after birth. As far as possible, goats and sheep were examined together, but unforeseen technical difficulties and, in some cases, scarcity of animals resulted in one species

receiving more attention than the other in different aspects of the work.

The physiological emphasis of the work centred upon several independent objectives related to or involving the use of catheterised preparations. Although there is some overlap between chapters, the following is a brief guide to the objectives and the presentation of material. Chapter Two deals with the general materials and methods. The main aim of the work described in Chapter Three was to determine the time required for animals to adapt to the laboratory, a subject which had received little attention until the present work. Although it is generally recognised that anaesthesia and surgery are stressful there is no direct evidence to indicate that acute procedures stress the mother. Evidence of maternal stress and a measure of the time required for postoperative recovery following surgical insertion of uterine catheters is given in Chapter Four. After taking into account the findings reported in Chapters Three and Four, the following investigations were made using conscious, catheterised animals. Firstly, an attempt was made to discover whether the differences in hormone balance during pregnancy between the goat and sheep were associated with differences in fetal fluid composition. Using established catheterisation techniques the compositions of the fetal fluids of the goat were examined and, in Chapter Five, compared with those previously reported for sheep (Mellor and Slater, 1971) and anaesthetised cattle (Reeves, Daoud, Gentry and Eastin, 1972). Secondly, a new catheterisation technique was developed to discover if modification of swallowed amniotic fluid occurred in the fetal rumen (Chapter Six). Thirdly, an

hypothesis, proposed by Mellor and Slater (1972a, 1974), relating changes in the electrolyte composition of allantoic fluid and fetal urine to changes in the plasma concentrations of fetal adrenocortical hormones in sheep, was investigated as a conjoint study (Chapter Seven). Finally, a non-surgical catheterisation method was devised to obtain frequent blood samples from neonatal animals in order to examine in detail the physiological changes occurring immediately after birth as indicated by changes in blood composition (Chapter Eight). As part of the introduction to Chapter Eight current views on parturition in sheep and goats are examined. The thesis ends with Chapter Nine which contains a physiological assessment of the chronic approach to fetal studies, drawing on the author's present work and the published experiences of other workers. It was not considered necessary to begin with a comprehensive literature review since relevant publications have been included in each section of work.

CHAPTER TWO

MATERIALS AND METHODS

Unless otherwise stated the procedures were as follows:

2.1 ANIMALS

2.1.1 Source

Thirty 1-9 year old goats (40-80 kg) were obtained from the Institute herd. The goats were of mixed breeding but many showed the characteristic markings of the British Toggenburg breed.

Twenty-five 4-6 year old Scottish Blackface sheep (40-70 kg) and two Welsh Mountain ewes (40-50 kg) which were brought to the laboratory from the Institute farms were used.

2.1.2 Mating

With the exception of 13 yearling goats all animals had given birth to and successfully reared young in the previous year. Mating took place between the beginning of October and the end of January in the goats and from November to January in the sheep. Entire males fitted with a raddle (Sire sine harness, Hortico Ltd., Victoria, Australia) were run continuously with the females which were checked daily for signs of mating. After mating the animals were run with a male for a further 22 days (goats) or 18 days (sheep) to check for any returns to service. The date of last mating was taken as day 0 when calculating gestational ages.

2.1.3 Housing

The Institute goat herd was housed in a covered yard. Twenty

to thirty days after mating the goats were transferred to the laboratory where they were kept in individual pens in a well ventilated animal house. Fourteen goats, studied in 1974, were housed on deep litter and 12 of the goats, studied in 1975, were kept on slats. Sheep were mated on the Institute farms and at 30-40 days gestational age were brought into individual slatted pens in an animal house.

The slatted pens were cleaned and if necessary hosed out daily after feeding and when required straw or sawdust was added to the deep litter pens.

Three to four days after parturition the goats and sheep were transferred to two large pens in a covered yard where they remained until the young were weaned at 6-8 weeks of age. All male offspring were castrated within one week (sheep) or 2-3 weeks (goats) of birth using a bloodless castrator.

2.1.4 Nutrition

The goats were fed as a group while in the Institute herd and each received about 0.5-0.7 kg pelleted feed/day (Ewebol pencils 302, BOCM Silcock) and 0.4-0.8 kg hay/day. On being brought into the animal house they were fed individually 0.8-1.6 kg pelleted feed/day (Ruminant A or B, Seafield Mill, Midlothian) given as one feed at 0830-1030 hr and 0.3-0.5 kg hay given between 1400 and 1600 hr. Similarly, sheep received 1.0-2.0 kg pelleted feed/day (Ruminant A or B).

Some of the goats and all sheep were weighed weekly. Each day before feeding a blood sample was taken from a jugular vein of each animal (Section 2.5.1) and the glucose concentration in plasma was determined (Section 2.7.6.d). The quantity of feed given to each animal was increased during pregnancy so that plasma glucose concentrations were maintained as far as possible above 500 mg/l and body weight increased by up to 10% by term. Unlimited water was available. Daily feed and water intakes were recorded for all animals until parturition.

During lactation goats and sheep were given pelleted (Ruminant A or B) ad libitum and 0.3-0.4 kg hay. From 3-4 days of age kids and lambs had access to creep feed (Ewebol lambwena 301, BOCM Silcock).

2.2 PREOPERATIVE PREPARATION

All animals were handled daily for 4-8 weeks before operation. Operations were performed at 81-111 days of gestation in goats or 85-121 days in sheep. At least two weeks before operation the sheep were completely shorn posterior to the last rib. Feed but not water was withheld for 48 hr prior to the operation and most animals were given parenteral injections of progesterone in oil. Progesterone treatment of the goats was varied and is described in detail in Chapter Five but sheep received 63 mg progesterone in oil (Organon Laboratories Ltd., Morden, Surrey) on the day before operation.

2.3 GENERAL OPERATIVE PROCEDURES

2.3.1 Preparation and sterilisation of equipment

The surgical theatre walls and floors were washed with an

iodine based disinfectant (Fam, Vanodine Ltd., Eccles, Manchester). Before use all instruments, drapes, operative clothing, glassware and syringes were sealed in boxes or autoclavable nylon bags and sterilised in an autoclave. Catheters (see Section 2.4.1 for details) were made up and sealed individually in plastic bags and sterilised by Gamma irradiation (Ethicon Ltd., Edinburgh). Stringent aseptic precautions were observed throughout each surgical operation.

2.3.2 Anaesthesia

Anaesthesia was induced and maintained with pentobarbitone sodium given intravenously through a catheter placed in a maternal jugular vein. Following endotracheal intubation the animals were secured in dorsal recumbency and breathed 100% oxygen in a closed-circuit to and fro system in which a soda-lime canister was incorporated to absorb carbon dioxide. The system was flushed out once or twice during operation to remove any nitrogen which might have accumulated.

2.3.3 Preparation of the operative site

The whole of the ventral surface of the abdomen posterior to the last rib and most of the right flank were clipped and washed with soap and water to remove dirt and grease. The whole area was then drenched with a succession of sterilising solutions: an iodine antiseptic solution (Pevidine, Berk Pharmaceuticals Ltd., Surrey), 0.05% $^{w}/_{v}$ chlorhexidine and 0.1% $^{w}/_{v}$ thiomersal solution (50:50 acetone:ethanol). The animal was then covered with sterile drapes.

2.3.4 General surgery at catheterisation

An oblique incision was made through the skin and muscle layers on the right side of the abdomen parallel to the fibres of the rectus abdominus muscle, extending approximately 15 cm postero-medially from the ventral border of the right subcutaneous muscle towards the mammary gland. This exposed the pregnant uterus (details of catheterisation methods are given in Section 2.4). After catheters had been inserted into the conceptus, the uterus was returned to the abdominal cavity as far as possible in its original position. The abdominal incision was closed with four layers of continuous catgut sutures. The anterior end of the skin incision was extended about 8 cm dorsally and closed over the catheters with silk sutures. The catheters protruded by 3-5 cm at intervals of 1-2 cm from the dorsal end of the incision.

2.3.5 Protection of catheters

A large bandage was used in both species to protect the catheters. In sheep the bandage, covering most of the ventral and lateral abdomen, consisted of two thicknesses of lint sewn together and tied in place over the animals back with strips of 7.5 cm bandage. In goats the bandage was modified to cover the thorax and abdomen completely and included a waterproof sheet of thick polythene between two of the three layers of lint. This generally prevented goats from reaching the catheters and gave added warmth.

2.3.6 Postoperative care

On completion of surgery the endotracheal tube and maternal jugular catheter were removed and the animals were placed in a pen

in a warm room (22-25°C) where they remained for 2-3 days (sheep) or 3-7 days (goats). Immediately after operation and for three days thereafter animals were given 20-25 mg/kg procaine penicillin with 20-25 mg/kg dihydrostreptomycin or 5-7 mg/kg oxytetracycline hydrochloride. Some animals also received the same dose at about 9 hr and 36 hr after surgery. When animals regained consciousness chopped hay and grass were offered. Pelleted feed was not given until at least 9 hr after operation as a precaution against choking. Saline solution (0.45% $^{w}/_{v}$) was offered in place of water for the first 1-2 days to help restore fluid and electrolyte balance. Greens and hay were sometimes given to encourage animals to eat during the first 2-3 days after operation. Skin sutures were removed after two weeks.

2.4 METHODS OF UTERINE CATHETERISATION

2.4.1 Types of catheter used

Amniotic and allantoic sac catheters were made by attaching a two-way Luer tap to the outlet tube of a Foley two-way balloon catheter - 41 cm long, size 12FG, with a 30 ml balloon cuff - (Medical Supplies Assoc., Dundee) (Mellor, 1970a).

Fetal bladder and peritoneal catheters were made from 40-50 cm of 0.63 mm internal diameter (i.d.) 1.4 mm outside diameter (o.d.) vinyl tubing (Portex Ltd., Hythe, Kent). Each catheter had a three-way Luer tap attached to one end and at the other end two small holes on opposite sides of the tubing about 0.4 cm apart between the tip and a cuff about 1.3 cm from the end. The cuff was made by tying silk thread firmly around the catheter (Mellor et al., 1972).

Fetal ruminal and abomasal catheters were indentical in design to fetal bladder catheters, but had diameters of 0.86 mm (i.d.), 1.5 mm (o.d.) or 1.0 mm (i.d.), 2.0 mm (o.d.).

Fetal vascular catheters were made from 60-85 cm of vinyl tubing (0.63 mm i.d., 1.4 mm o.d.) with a three-way Luer tap at one end and 10 cm of smaller diameter vinyl tubing (0.5 mm i.d., 0.9 mm o.d., or 0.4 mm i.d., 0.8 mm o.d.) joined to the other end. In addition, 2 mm of vinyl tubing (1.4 mm i.d., 1.9 mm o.d.) was fixed over the junction to act as a retaining cuff (Mellor and Matheson, 1975).

Neonatal umbilical catheters were made by attaching a two-way tap to the outlet tube of an infant umbilical cannula - 35 cm long, size 6FG - (Portex Ltd.).

2.4.2 Established catheterisation techniques

The amniotic and allantoic sacs were catheterised as described by Mellor (1970a). A small incision was made in the uterus and fetal membranes and the catheter was inserted and held in place using a double (amniotic) or single (allantoic) silk purse-string suture. The retaining cuff around the catheter was inflated with 15-20 ml of sterile 0.9% saline solution. The amniotic sac was catheterised through the amniochorion on the antimesometrial surface of the uterus. The allantoic sac catheter was inserted into the tip of the uterine horn. Speed was essential in catheterising the allantoic sac as contraction of the tip of the uterine horn usually occurred after the sutures were placed in the uterine wall.

The fetal bladder was catheterised as outlined by Mellor et al. (1972). Briefly, the hind-end of the fetus was drawn gently through a 4-5 cm incision in the uterus and amniochorion with minimum loss of amniotic fluid. A catheter was placed in the bladder after a small midline incision had been made in the fetal abdomen. The catheter was secured with a single purse-strong suture so that the catheter cuff was included in the tie. Care was taken to avoid the umbilical arteries which are in apposition to the lateral surface of the bladder.

The fetal peritoneal cavity. After a fetal bladder catheter had been tied in place, an additional identical catheter was placed in the fetal peritoneal cavity of some animals. It was secured, together with the bladder catheter, by sutures used to close the fetal abdominal incision.

The fetal umbilical vessels were catheterised as outlined by Mellor and Matheson (1975). Main umbilical arterial branches and venous tributaries of cotyledons at each end of the conceptus were located by gentle palpation through the uterine wall ventro-laterally in the body of the uterus (singleton pregnancies) or in the lesser curvature at the junction of the appropriate horn and the body of the uterus (bicornuate twin pregnancies). The small diameter part of the catheter was cut to about 3 cm and the catheter was threaded into a small tributary and secured so that its tip lay about 5 mm inside the major vessels.

2.4.3 New catheterisation techniques

The fetal rumen and abomasum. A 4-5 cm incision was made in the uterine wall and fetal membranes and the hind legs and abdomen of a fetus were drawn through to expose the lateral area immediately posterior to the last rib on the left side. A 1.5 - 2.0 cm incision was made through the abdominal wall parallel to and 0.5 cm from this last rib, extending dorsally to a point 1.5-2.0 cm from the spinal column. The reticulo-rumen, which was identified by its rounded appearance, protruded through the incision when gentle pressure was applied to the abdomen close to the incision. The abomasum, which was long and tapered at the pyloric end, was usually located posterior to the ventral end of the incision. After the rumen had been catheterised the abomasum was drawn through the incision and catheterised 2-3 cm from the pylorus. At operation the reticulo-rumen and the abomasum each contained about 10-15 ml of fluid.

The aortae and venae cava of the neonate were catheterised non-surgically via the cut umbilical vessels. This technique was devised conjointly with Dr.D.J.Mellor in this laboratory. Close watch was kept on animals before parturition. At birth care was taken to avoid stretching the umbilical cord which was cut 6-8 cm from the umbilicus immediately the fetus was delivered. The cord was held firmly to reduce contraction of the vessels while the cut end of an artery or vein was located. An umbilical catheter was passed into the lumen of one vessel in each neonate a distance of about 7 cm (vein) to 15 cm (artery) so that the catheter tip lay in the vena cava or aorta. The catheter was held in place with a silk ligature which on arteries in particular had to be firmly tied.

Veins were more difficult to catheterise than arteries due to the flaccid nature of the walls. Although rapid contraction of the arteries occurred at birth they could be readily stretched again before catheterisation. With practice the first catheter blood sample could be taken within two minutes of birth. A cotton bandage (7.5 cm wide), soaked in amniotic fluid to prevent rejection by the mother, was wrapped around the body of the newborn. The catheter was tucked firmly under the bandage out of reach of the mother. The bandage was loosened when necessary after the newborn had sucked. At the end of the experiment, the catheter was removed and the cord tied with silk thread. The newborn then received 125 mg procaine penicillin and 125 mg dihydrostreptomycin subcutaneously and was closely observed over the following week.

2.5 SAMPLING PROCEDURES IN PREGNANCY

All sampling was carried out daily before the animals were fed. The animals generally stood quietly, frequently cudding and were apparently unstressed by sampling procedures.

2.5.1 Maternal jugular blood

Blood samples (4-6 ml) were obtained from a jugular vein into heparinised 10 ml vacutainers. These contained 0.5 mg dry heparin.

2.5.2 Fetal fluid and umbilical blood

All syringes (5 ml, Plastipak, Becton Dickinson and Co., Ireland) were packed individually in autoclavable nylon bags and sterilised in an autoclave before use. Sterility was maintained throughout. The catheters and surrounding skin were drenched with 0.1%^{w}/v

thiomersal solution (50:50 acetone:ethanol). To avoid contamination of samples with thiomersal solution the following method was adopted when sampling the fetal fluids: 0.5 ml of fluid was withdrawn into a syringe and discarded. A further sample (about 1 ml) was taken into another sterile syringe and was kept for analysis. Fetal umbilical catheters were sampled according to Mellor and Matheson (1975). After cleaning with thiomersal the catheter was flushed with a pulse of 0.5 - 1.0 ml of 0.9% $^{w}/_{v}$ saline solution (volume of catheter and tap = 0.5 - 0.7 ml). Fetal blood (0.75 ml) was withdrawn into the same syringe and was discarded. A 3.0 ml blood sample was drawn into a fresh syringe containing 0.25 mg dry heparin. The catheter was then flushed with 3.0 ml of 0.9% saline followed by 0.75 - 1.0 ml heparin saline solution (3 mg heparin/0.9% saline solution). After a blood or fluid sample had been taken, dead space air was expelled immediately from the syringe which was then capped ready for pH determination. On completion of sampling all catheter taps and the incision were washed with thiomersal solution. Cleaning the incision reduced the chances of a subcutaneous infection around the site of catheterisation.

One or two drops of fluid from each sample were transferred to a blood agar plate and incubated for 48 hr at 37°C to test for bacteria. If an infection was detected antibiotics (25 mg/kg procaine penicillin and 25 mg/kg dihydrostreptomycin or 7 mg/kg oxytetracycline hydrochloride) were given as parenteral injections twice daily. In extreme cases 500 mg ampicillin sodium was flushed daily directly into the fluid sac via the catheters.

2.5.3 Infusions

Each pen was divided with a partition to restrain the animal so that it could stand up and lie down but not turn around. The animal was restrained in this way 3-4 days before the infusion began. Prior to this the pen had been divided at regular intervals so that restriction was not a totally new experience to the animal.

Precautions were taken to maintain sterility when setting up infusion lines. Each infusion line (7-10 m vinyl tubing 0.9 i.d., 1.5 o.d.; Portex Ltd.) was sterilised by rinsing with 0.1% $^{w}/_{v}$ thiomersal solution (50:50 acetone:ethanol) and was attached to a 50 ml syringe containing the infusate. The syringe was connected to a calibrated pump (syringe pump SP/15; Scientifica and Cook Electronics Ltd.) which was placed on a shelf above the animal. Residual thiomersal solution and air were expelled from the line with sterile isotonic saline solution before it was attached to the umbilical vein catheter.

2.5.4 Removal of catheters at parturition

Immediately after birth the balloon cuff on the amniotic and allantoic sac catheters was deflated and within one or two days the catheters could be gently pulled out through the flank incision. The vinyl catheters in the fetal bladder, peritoneal cavity, rumen and abomasum pulled out of the fetus during birth and could then be removed easily from the mother. The point of entry of the catheters through the skin closed within days leaving little trace of the catheterisation.

2.6 SAMPLING PROCEDURES IN THE NEONATE

2.6.1 Sampling of umbilical cord catheters

The catheter was filled with heparin saline solution (1 mg/ml 0.9% $^{w}/_{v}$ saline solution) between sampling. The newborn was held gently near the mother and 0.75 ml of blood was withdrawn into a 5 ml syringe and discarded to remove the heparin saline solution from the catheter. A further 2-3 ml of blood was withdrawn into a syringe containing 0.25 mg dry heparin. Any air was immediately expelled from the syringe which was then capped. The catheter was refilled with heparin saline solution and the newborn was returned to the pen after checking the protective bandage.

Sample syringes were immediately placed in iced water before blood gas analysis after which the blood was centrifuged and the plasma obtained stored at -20°C in sealed containers before analysis.

2.7 ANALYTICAL METHODS

In general the relevant publication on each analytical procedure is given with a brief description of the basis of each method. All analytical procedures were carried out by the author except those for fructose, lactate and thyroxine.

2.7.1 pH of fluid and urine

The pH was measured using a micro-electrode attached to a pH meter (model 27, Radiometer, Copenhagen). A small total immersion electrode attached to the same pH meter (combined electrode, Radiometer) was used to measure the pH of the more viscous ruminal and abomasal fluids.

2.7.2 pH, PO_2 and PCO_2 of blood

The pH and the partial pressures of oxygen and carbon dioxide (PO_2 and PCO_2, respectively) were measured with an ultra-micro pH/blood gas system (I.L.113, Instrumentation Laboratories (UK) Ltd., Altrincham, Cheshire) maintained at 39.5°C. The pH, oxygen and carbon dioxide electrodes were calibrated daily before use and after analysis of the samples.

2.7.3 Packed cell volume (PCV)

PCV was determined using a micro-haematocrit centrifuge (MSE, Crawley, Sussex).

2.7.4 Osmolality

Osmolality was measured by freezing point depression (Precision Osmometer, Precision Systems, USA). The freezing point of a solution is a measure of its solute concentration since a 1 mOsm/kg water change in concentration causes a change of 0.00185°C in the freezing point. Aqueous salt solutions of 100 and 500 mOsm/kg water (Precision Systems) were used for calibration as sample concentrations were generally within these limits. The osmometer was calibrated before and checked after use and every tenth sample measurement was made in duplicate. Variation between duplicates was never more than 4 mOsm/kg water.

2.7.5 Sodium (Na) and potassium (K)

Concentrations of Na and K were determined using a flame photometer (I.L.343, Instrumentation Laboratories (UK) Ltd.). This incorporated lithium as an internal standard. The samples and

lithium standard (3000 mmol/l) were diluted 1:200 with distilled water in an automatic dilutor system attached to the flame photometer before analysis. Aqueous solutions containing 145Na/5K mmol/l or 100Na/100K mmol/l (Instrumentation Laboratories (UK) Ltd.) were used as standards. The machine was checked against the standard after every 10 samples.

2.7.6 Colorimetric methods

These involved a change in absorbance within the visible light range (400-700 nm) and made use of automated flowlines.

Flowlines consist of units (e.g. mixing coils, dialyser, heating coils, delay coils and colorimeters) linked together by glass tubing through which reagents are pumped continuously by a peristaltic pump. A sampler unit places samples in turn below a sampling probe which takes up the required amount of sample. Samples pass successively along the tubing at a set time interval. Air bubbles introduced into the system in a regular pattern reduce carry-over by preventing the samples from diffusing into each other and also scour the walls of the tubing. Between samples a distilled water wash was taken up by the sample probe. The wash period was varied to reduce carry-over to an acceptable level in each method. In general a sampling time of 20 secs sample: 40 secs wash was used. The absorbances of standards and unknowns were recorded as peaks on a moving chart recorder attached to the colorimeter. Peak heights were measured using a pencil follower (Dmac, Cetac Systems Ltd., Glasgow) which determined the X and Y coordinates of the peaks. The digital output of the pencil follower

was recorded on punch tape and the tape was fed directly into a PDP 8/f computer.

a. Chloride (Cl)

Cl concentrations were determined using the method of Zall, Fisher and Garner (1956). This assay is based on the principle that when chloride ions react with mercuric thiocyanate to form mercuric chloride, thiocyanate is released. Thiocyanate forms a red complex $Fe(SCN)_3$ with ferric ions. The sample was diluted and dialysed with an aqueous solution of sulphuric acid (1.6%). The reagents used were a diluent of 1.6% sulphuric acid, containing 1 ml/l Hyamine 2389 as a wetting agent, and a colour reagent containing mercuric thiocyanate and ferric nitrate. A limited amount of aqueous 7% $^{w}/_{v}$ mercuric nitrate solution was used to adjust the intensity of the coloured complex. Aqueous solutions (10-150 mmol/l) of sodium chloride (BDH Chemicals Ltd., Poole, Dorset) were used as standards.

b. Urea

Urea nitrogen concentrations were determined according to Marsh, Fingerhut and Miller (1965). If urea is heated with substances containing two adjacent acetyl groups coloured compounds are formed. Diacetyl monoxime was used under acid conditions. The presence of thiosemicarbazide intensifies the colour of the reaction product formed enabling the determination to be run without using concentrated acids. The reagents used were an acid solution, containing phosphoric acid, and a colour reagent, containing diacetyl monoxime and thiosemicarbazide. 1 ml/l of a wetting agent (Brij-35) was added to each solution. Aqueous solutions (50-500 mg/l) of urea (BDH) were used

as standards.

c. Iorganic phosphorus (P)

P concentrations were determined according to Robinson, Roughan and Wagstaff (1971). The sample was diluted and dialysed with an aqueous sulphuric acid solution (1%). P in the dialysate couples with a molybdivanadate reagent to form a coloured compound. The reagents used were a diluent of 1% sulphuric acid, containing 1 ml/l octan-2-ol as a wetting agent, and a colour reagent, containing ammonium molybdate and ammonium vanadate in nitric acid. Aqueous solutions (20-100 mg/l) of potassium orthophosphate (BDH) were used as standards.

d. Glucose

Glucose concentrations were determined using the glucose oxidase method of Trinder (1969). Hydrogen peroxidase is one of the products of oxidation of glucose by glucose oxidase. On treatment with peroxidase H_2O_2 yields O_2 which oxidases a suitable colourless substrate (phenol in the presence of 4-amino phenazone). The reagents used were a diluent, containing 4-amino phenazone, and a colour reagent containing glucose oxidase, peroxidase and phenol. Azide was added as a preservative to both solutions. Dialysis was used to remove sample proteins. Aqueous solutions (200-1000 mg/l) of D-glucose (BDH) were used as standards.

e. Fructose

Fructose concentrations were determined by a modification of the method devised by Roe (1934) based on the Seliwanoff reaction.

When fructose is dehydrated with hydrochloric acid in the presence of resorcinol a red coloured product is formed. The use of concentrated HCl overcame the need to use ethyl alcohol as the solvent medium. At 80°C no colour is produced by glucose making determination of fructose possible without interference from glucose. The reagents used were concentrated HCl and a 0.1% $^{w}/_{v}$ aqueous solution of resorcinol. Brij-35 was added to the saline diluent and the resorcinol solution as a wetting agent. Aqueous solutions (500-8000 mg/l) of D-fructose (BDH) were used as standards.

f. Lactate

Lactate concentrations were determined by the method of Hochella and Weinhouse (1965). Lactate is oxidased to pyruvate in the presence of lactic dehydrogenase with a reduction of NAD^{+}. NADH is oxidased by diaphorase and the dye 3-p-nitrophenyl-2-p-iodophenyl-5-phenyltetrazolium chloride (INT) is reduced to the corresponding formazan which is strongly absorbed at 500nm. Samples were diluted with an aqueous solution of 1.6% $^{w}/_{v}$ sodium sulphate and dialysed with 1.6% $^{w}/_{v}$ $NaSO_4$, containing 20 ml/l Triton X-100. The other reagents used were a glycine buffer solution (pH 9.6), an enzyme-cofactor solution (containing diaphorase, NAD^{+} and lactic dehydrogenase) and the INT dye solution. Aqueous solutions (100-2500 mg/l) of L-lactic acid (BDH) were used as standards.

2.7.7 Calcium (Ca) and Magnesium (Mg)

Ca and Mg concentrations were determined using atomic absorption spectrophotometry by the methods of Trudeau and Freier (1967) and Stewart, Hutchinson and Flemming (1963) respectively. All standards

and unknowns were diluted 1/25 with an aqueous solution of lanthanum chloride (6.5 ml/l of a stock $LaCl_3.7H_2O$ solution containing 10% $^{w}/_{v}$ La). Aqueous solutions of $CaCl_2$(80-1500 mg/l) and $MgCl_2$ (10-80 mg/l; BDH) were used as standards.

2.7.8 Quality control

Quality control for Cl, urea, P, glucose, fructose, lactate, Ca and Mg determinations consisted of checks performed by the computer of various criteria including the regression coefficient and residual variation of the best line through the standards. Except for lactate determinations aqueous standards were corrected against versatols (pooled human serum standardised for several constituents; W.R.Warner, Eastleigh, Hampshire) and the factor by which the estimated standards were multiplied as a result of the correction was printed out. Lactate concentrations were corrected against plasma containing a known amount of lactate made up to 500 mg/l with L-lactic acid (BDH).

The use of correction factors was considered to provide adequate quality control between batches. The versatol correction was included when batches of fetal fluids were determined although fetal fluid protein concentrations are low (Hervey and Slater, 1968). Within-batch quality control was monitored by assaying samples of pooled caprine or ovine plasma after every 10 samples. Baseline drift within batches was observed from the changes in pool plasma concentrations and also by the changes in initial and final baseline versatol values. Samples were usually analysed in batches of 64 samples. A typical run consisted of five standards, a distilled

water blank, versatol, four unknowns, pooled plasma, 60 unknowns with a pooled plasma after every 10 unknowns, blank, versatol.

2.7.9 Hormone assays

All radioactive substances were obtained from the Radiochemicals Centre, Amersham, Buckinghamshire.

Protein binding assays

The first two procedures utilise the relatively specific steroid binding properties of corticosteroid binding globulin (CBG) and the ability of unlabelled steroids to displace tritiated corticosteroids of high specific activity from the CBG. This technique was first used by Murphy (1967). Specificity depends largely on the fact that only a restricted number of steroids compete for binding sites on the CBG.

a. Progesterone

Progesterone concentrations were determined by a modification of the protein binding technique (Bassett and Hinks, 1969) as described by Thorburn Bassett, and Smith (1969) and Challis, Heap and Illingworth (1971). The CBG used in all assays came from human plasma. The isotope-CBG solution was made up 24 hr before each assay as follows and stored at 4°C until use: 100 µl of 1,2 [^{3}H] corticosterone (0.5 µ Ci) and 200 µl CBG was made up to 100 ml in Tris/EDTA buffer (0.1M Tris, 0.001M EDTA).

Plasma samples (0.4 ml) were extracted with 5 ml petroleum ether (40-60° b.p.). Double distilled water (0.5 ml) was added to facilitate removal of the more polar adrenal steroids. Extraction

tubes were then rotated on a mixer for 30 min. The aqueous layer was frozen by standing the tubes in a methanol bath (-60°C) for 10-15 min. The organic phase was decanted into assay tubes and dried down at 45°C under a stream of air.

Dried plasma extract was incubated at 45°C for 10 min with 1 ml of the isotope-CBG solution. The reaction was brought to equilibrium by placing the assay tubes in an ice bath for 10 min. Free and bound corticosterone were separated using dextran charcoal. 0.5 ml of 0.5% $^{w}/_{v}$ gelatin and 0.5 ml dextran charcoal (0.025% $^{w}/_{v}$ dextran plus 0.25% $^{w}/_{v}$ charcoal (Norit A)) was added to each tube at 4°C. All tubes were centrifuged at 2000g for 30 min and 0.4 ml of the supernatant was removed into 3 ml of scintillation fluid (Aquasol, New England Nuclear Corporation, Boston, USA) for counting. Aqueous solutions (0.5, 0.75, 1.0, 2.5, 5.0, 7.5 and 10 ng/ml) of progesterone (Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire) were used as standards.

The efficiency of extraction ($\pm$SE) of 28 samples in seven assays was 89$\pm$1.3%. The recovery of 5 ng progesterone added to 0.4 ml plasma from an ovariectomised goat was 4.55$\pm$0.4 (14) ng/ml. A distilled water blank gave a concentration which never exceeded 0.25 ng/ml. Samples from a goat obtained on one day in pregnancy were included in every assay and gave a mean concentration ($\pm$SE) of 6.5$\pm$0.2 ng/ml (seven assays) and an interassay coefficient of variation of 18.2%.

b. Corticosteroids

Initially, corticosteroid concentrations were measured by a modification of the protein binding technique of Bassett and Hinks (1969) described by Mellor, Smith and Matheson (1975b). In this method gel filtration on a small column of Sephadex was used to separate protein bound [^{3}H] corticosterone from free [^{3}H] corticosterone. A sample of pooled ovine plasma in each assay gave a mean ($\pm$SE) concentration of 14.8$\pm$0.45 ng/ml in 24 assays and an interassay coefficient of variation of 14%.

Further modifications were made and later determinations were carried out using the following procedures: endogenous corticosteroids were removed from 10 ml of dog plasma in a Sephadex G25 (coarse) column at 45°C. The dog plasma was eluted with 0.05M borate buffer, the first 50 ml was discarded and the next 35 ml containing the plasma protein was collected and made up to 50 ml with buffer. Aliquots (4 ml) of this CBG solution were stored at -20°C and made up to 175 ml with borate buffer immediately before use.

A plasma sample (0.1 ml) was precipitated with 0.9 ml ethanol. 0.1 ml of the supernatant was dispensed into an assay tube with 0.2 ml 1,2 [^{3}H] cortisol solution (0.06 μCi/ml) and freeze-dried. CBG solution (1ml) was added and the tubes were incubated at 45°C for 20 min. The reaction was brought to equilibrium by placing the tubes in iced water for 30 min. Free and bound corticosteroid was separated by the addition of 0.4 ml dextran charcoal solution (0.04% ^{w}/v dextran and 0.4% charcoal (Norit A) at 4°C and all tubes were stood for 30 min in an ice bath before being centrifuged at

2000g for 30 min. Supernatant (1ml) was dispensed into 10 ml of scintillation fluid (toluene containing 0.3% $^{w}/_{v}$ terphenyl and 0.01% $^{w}/_{v}$ POPOP) and counted. Standard dilutions (5-70 ng/ml) of cortisol (Koch-Light Laboratories Ltd.) were prepared and made up in corticosteroid free ovine or caprine plasma. Within-assay drift was monitored by using two batches of standards in each assay. Pooled ovine plasma in each assay gave a mean ($\pm$SE) concentration of 84.6$\pm$2.1 ng/ml in three assays and an interassay coefficient of variation of 6.5%.

Radioimmunoassays

c. Luteinizing hormone (LH)

LH was determined by a double antibody radioimmunoassay (Carr and Land, 1975). A specific rabbit (anti-ovine LH) antiserum and a purified preparation of ovine pituitary LH labelled with ^{125}I were used. Rabbits had been immunised with horse IgG to provide the precipitating antiserum. The assay measures LH in ovine plasma, but the sensitivity of the assay in caprine plasma was unknown. It was not possible to evaluate the effect of caprine plasma on the assay using LH-free plasma containing added exogenous LH since no hypophysectomised goat plasma was available. Plasma effects in sheep were small when assays were carried out in a diluent containing bovine serum albumin (Carr and Land, 1975). Therefore, in an attempt to compare the effects on the assay of caprine relative to ovine plasma the following test was carried out. Plasma from an ovariectomised goat and sheep, having high LH concentrations, were diluted in varying amounts with buffer, containing bovine serum albumin, and assayed. The gradients of the two respective dose-

response curves (activity vs concentration of plasma/sample) were similar. So, it was assumed in this study that plasma effects in the goat and sheep were similar. While it is likely that the assay measured an ovine LH like compound in caprine plasma further tests (e.g. the use of purified caprine LH and LH free plasma) would be necessary to establish the exact nature of the substance in caprine plasma attaching to the antibody binding sites of the antiserum. Samples were assayed at a 40% dilution. Concentrations of 0.08, 0.16, 0.20, 0.32, 0.40, 0.64, 0.80, 1.25, 2.58 and 5.12 ng/ml NIH-LH-S18 (Endocrine Study Section, National Institute of Health, Bethesda, USA) were used as standards. Between-assay drift was monitored by using two batches of standards in each assay. All plasma samples from one goat were measured in one assay. Samples of pooled plasma were included in each assay. Between-assay variation was 12%.

d. Thyroxine (T_4)

Throxine concentrations were measured according to Seth, Rutherford and Mckenzie (1975) by a solid-phase radioimmunoassay. Anti-ovine thyroxine antiserum is covalently coupled to micro-crystalline cellulose. The insoluble solid-coupled antibodies so produced permit separation of antibody-bound from free hormone by centrifugation. Interference from the thyroxine binding serum proteins on the antigen antibody reaction is virtually eliminated by the addition of 8-anilino-1-naphthalene sulphonic acid and incubation at a high pH (10.5). The label used was 3,5 ^{125}I-L-thyroxine. Dilutions (40, 60, 80, 160, 240, 360 ng/ml) of a stock L-thyroxine sodium salt solution (BDH) prepared in thyroxine free

ovine plasma were used as standards. Thyroxine free plasma was prepared by charcoal extraction. An aliquot from a plasma pool of thyroxine run at three different dilutions was used as a control in each assay. Mean ($\pm$SE) concentration of thyroxine in the control plasma in nine assays was 71.2 ± 1.2 ng/ml. Between-assay variation was 5.0%.

The results from LH and thyroxine assays were computed using a program devised from that of Rodbard and Leward (1970), which gave the estimated potency and 95% confidence limits for each sample. Some of the tests made in the course of analysis were for the linearity of the unknown curve and for parallelism of the unknown and standard curve.

2.8 PRESENTATION OF DATA

Where possible all results are given as mean values with standard deviation (SD) unless otherwise indicated. The number of observations (n) included in each mean value is given in brackets. The following analyses were adopted: Student's t test of independent samples for groups of equal or unequal size, the correlation coefficient test of significance and a covariance analysis (Snedecor and Cochran, 1967). Probability is given in brackets where the particular test shows a significant ($P < 0.05$) difference between means.

CHAPTER THREE

SOME BEHAVIOURAL AND PHYSIOLOGICAL CHANGES IN PREGNANT GOATS AND SHEEP DURING ADAPTATION TO THE LABORATORY

3.1 INTRODUCTION

One of the main purposes of the use of chronic techniques is to allow observation of the fetus and mother after the stress effects of surgery have passed. Therefore, catheterised animals should also be accustomed to their surroundings and experimental procedures otherwise, as suggested by Mellor and Slater (1973a), "difficulties of interpretation of results may ensure." In most laboratories animals are brought into experimental rooms directly from the field. In the field, the animals are in a group or flock, free to range, with continuous access to food of some sort, and usually have little contact with man. In contrast, in the laboratory the animals are generally penned individually, fed once or twice daily and are in close contact with the experimenter. At first most animals are highly excitable and require time to adapt to their new surroundings.

In the laboratory in which the present work was conducted, sheep to be used for chronic studies of the fetus have usually been allowed 6-8 weeks to adjust to the new conditions before experiments begin (Mellor and Slater, 1973a), while other workers have allowed sheep a minimum of 4-14 days (Buddingh et al., 1971; Comline and Silver, 1970). However, sheep have not been monitored in detail during this period and apart from a limited study by Block (1958) on the effects of handling and eating on plasma electrolytes there

is apparently no information on adaptation of goats to a new environment. In view of the above observations and the importance of ensuring that chronically catheterised animals have adapted to their environment, behaviour and physiological changes in both goats and sheep have been examined during their first 6-8 weeks in the laboratory.

The process of adaptation may be regarded as having two components: (1) adaptation to experimental rooms, a new diet and management procedures, and (2) adaptation to being handled directly during repeated blood sampling and other prolonged procedures. An attempt is made to differentiate between effects of these two components, and the time required for animals to adapt to the laboratory is also assessed.

3.2 EXPERIMENTAL METHODS

3.2.1 Animals

Eight goats and twelve sheep were brought into individual pens in the laboratory 30-40 days after mating. Four ewes each had an exteriorised carotid loop (surgically produced 18 months previously).

3.2.2 Degree of tameness

Four of the goats, designated 'tamed' were bottle reared and were accustomed to being handled, while the other four 'untamed' goats were used to seeing attendents but not to being handled. The four sheep with carotid loops had become used to handling during a three month period which ended six months before the start of the present experiment. During the six months they were kept as a

group in a covered yard and were not handled. These four sheep were described as 'partially tamed'. The other eight sheep were brought in directly from the field and were not accustomed to being handled.

3.2.3 Handling and criteria of tameness

All animals were handled daily for 5-10 min. This consisted of stroking their necks and heads and quietly talking to them. Untame behaviour was characterised by the animals remaining alert at the back of the pen or rapidly circling or attempting to jump out of the pen when approached. When handled directly untamed animals displayed extreme muscular tension and responded to stroking by attempting to escape, or by butting and stamping. When tamed these characteristics were completely absent; when approached for blood sampling the animals would come to the front of the pen and stand quietly often cudding while being held for sampling.

3.2.4 Heart rate measurements

In the four ewes with carotid loops heart rate was measured for one minute using each of the following procedures: heart rate 1 (HR_1) - counted from outside the pen by observing pulses in the carotid loop; heart rate 2 (HR_2) - counted immediately after entering the pen while gently holding the carotid loop; and heart rate 3 (HR_3) - counted after a further 3-5 min of talking to and gently stroking the ewe. Heart rates were measured at 0830-0900 hr before daily laboratory activities started and before the animals were fed, since heart rate increases markedly during feeding (Blair-West and Brook, 1969; Hays and Webster, 1971; Christopherson

and Webster, 1972). A significant ($P < 0.001$) post-prandial increase in heart rate was also observed in the four ewes in the present study after they had been in the laboratory for 45 days; mean ($\pm$SD) pre-feeding HR_1 was 63 ± 2 beats/min (8), postfeeding HR_1 (2 hr after the animals were given their daily ration was 91 ± 5 beats/min (8).

3.2.5 Blood sampling

Daily maternal samples were taken quickly (within 10-15 sec of entering each pen) before the glucose and corticosteroid concentrations in jugular blood could be affected by the sampling procedure. Repeated venepuncture experiments were performed at weekly intervals for five weeks. In each experiment blood was sampled at 0,7,15,30,45,60 and 90 min.

Results are expressed as mean $\pm$SD.

3.3 RESULTS

3.3.1 Experiment 1

It took up to one week before the goats ate the whole of their daily ration of pelleted feed. The behaviour of the four tamed goats did not change, but the four untamed goats became progressively less excitable, until after 2-3 weeks their behaviour was indistinguishable from that of the tamed group. Plasma glucose and corticosteroid concentrations were elevated in the untamed group during their first 5-9 days in the laboratory. The concentrations then decreased to values which were similar to those maintained by the tame group throughout the 28 day sampling period (Fig.3:1). The downward trend in glucose concentrations in both groups from the tenth day was probably due to increased fetal energy requirements and was checked

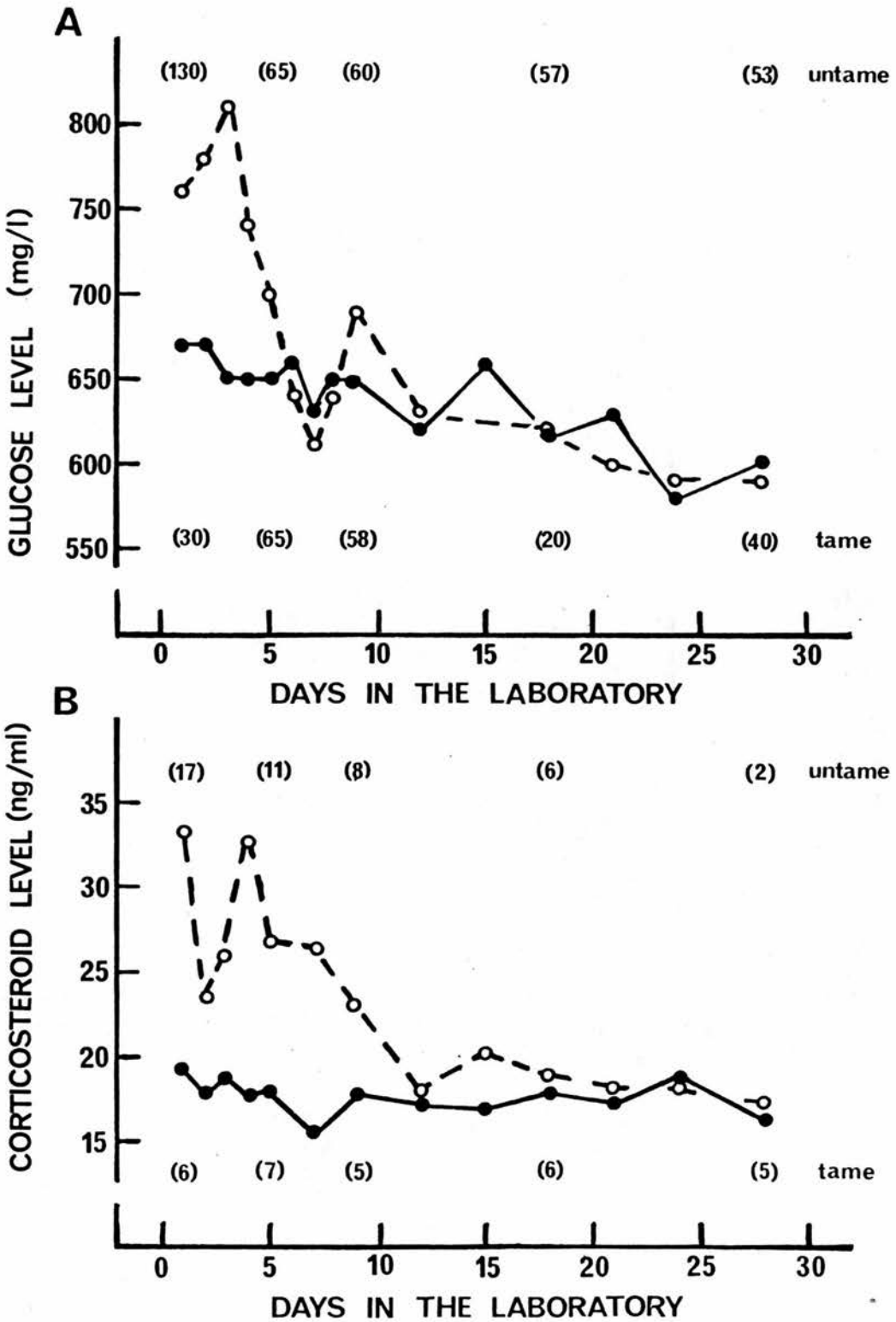

Figure 3:1

Changes in mean plasma concentrations of glucose (A) and corticosteroids (B) in four tame (●) and four untame (○) pregnant goats during their first 28 days in the laboratory. Standard deviations are given in brackets.

TABLE 3:1

Mean^{+}-SD corticosteroid concentrations (ng/ml) in plasma from eight untame sheep handled daily and bled weekly from the first day (group A) or after 14 days (group B) in the laboratory. The significence of differences between group A and group B are given.

		Days in the laboratory					
Sheep	n	1	8	15	22	29	36
Group A	4	28 ± 13	19 ± 4	19 ± 3	22 ± 2	17 ± 3	16 ± 2
Group B	4	-	-	19 ± 4^{ns}	17 ± 6^{ns}	15 ± 4^{ns}	16 ± 4^{ns}

^{ns} not significant.

on day 38 by an increase in the amount of feed offered. No consistent differences in plasma osmolality (range 278-294 mOsm/kg water) or Na (139-150 mmol/l) or K (3.8-4.8 mmol/l) concentrations were observed between and within the two groups.

3.3.2 Experiment 2

The eight untamed sheep in this experiment were extremely excitable at first and only became tame by the fifth or sixth week. Four of the untamed sheep (group A), handled and bled weekly from their first day in the laboratory, had high corticosteroid concentrations (mean 28 ± 13 ng/ml (4)) during the first weekly sampling. In four other untamed sheep, similar concentrations were maintained for the first four days in the laboratory (30 ± 12, 25 ± 16, 29 ± 14, 22 ± 9 ng/ml (4) , respectively, Mellor, unpublished data). During the following five weeks corticosteroid concentrations were consistently lower in group A and were not significantly different from those of the other four sheep in this experiment (group B), which were not bled and handled until they had been in the laboratory for two weeks (Table 3.1). Therefore, the higher plasma corticosteroid concentrations in sheep during their first week in the laboratory were probably due more to the change of environment than to blood sampling and handling procedures. Plasma glucose concentrations (range 540-690 mg/l) showed no consistent trend in either group and all animals ate the whole of their daily ration of pelleted feed within a week of entering the laboratory. No consistent differences between or within each group were observed in osmolality and the concentrations of Na or K (concentration ranges were 278-290 mOsm/kg water, 141-147 and 4.9-5.3 mmol/l, respectively).

3.3.3 Experiment 3

The four ewes in this experiment were those used in experiment 2 which were bled and handled from the day they entered the laboratory (day 1). In all animals repeated venepuncture was associated with a transient rise in plasma corticosteroid concentration, the magnitude and duration of which decreased each week until day 36 when little change was observed (Fig.3:2). These animals became tame between days 29 and 36. Although similar changes in corticosteroid concentrations during repeated blood sampling have been reported (Bassett and Hinks, 1969; McNatty and Young, 1973) the period of taming required for the transient rise to disappear in individual animals was not assessed and in some cases the animals had access to food (McNatty and Young, 1973). Except on day 1 when mean plasma glucose concentrations increased from 610 ± 20 mg/l to 770 ± 150 mg/l over the 90 min, glucose values showed no consistent change (range of mean concentrations 560-670 mg/l). However the between-animal variation decreased from 70-90 mg/l on day 1 to 10-30 mg/l on day 36. In contrast to goats subjected to repeated venepuncture (Block, 1958), there was no transient increase in plasma Na or decrease in plasma K concentrations in sheep during this procedure.

3.3.4 Experiment 4

Heart rate measurements began on the fifth day (day 5) the four ewes were in the laboratory and before this they were not handled. The basal heart rate (HR_1) of the four ewes decreased during the first 2-4 days of observation and thereafter showed small daily fluctuations (Fig.3:3). In all animals during the first 2-3 weeks of measurement, when the pen was entered heart rate increased. This

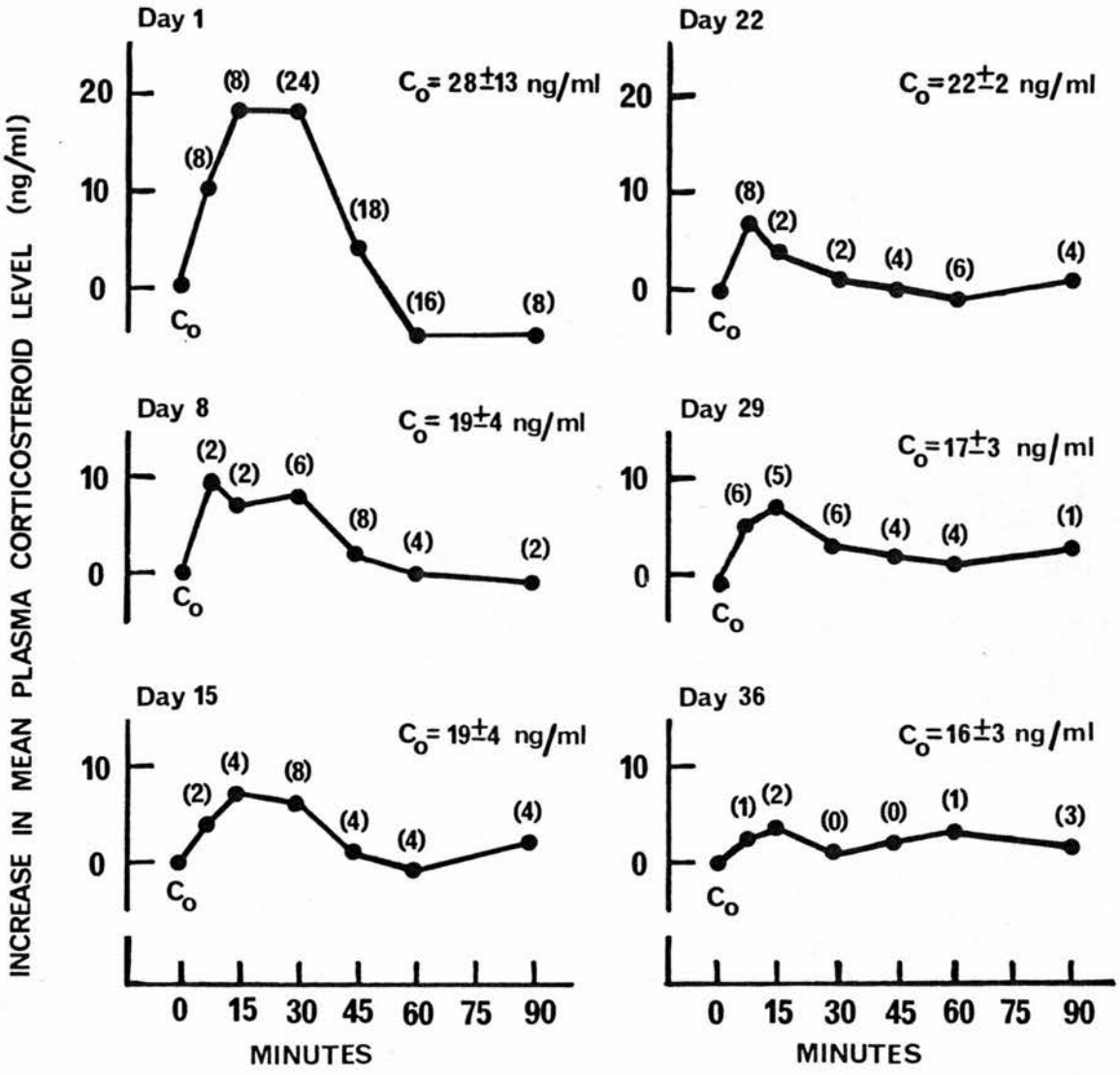

Figure 3:2

Mean changes in plasma corticosteroid concentrations in four sheep during repeated venepuncture experiments carried out at weekly intervals during the first five weeks the animals were in the laboratory. C_o = mean corticosteroid concentration at time 0. Standard deviations are given in brackets.

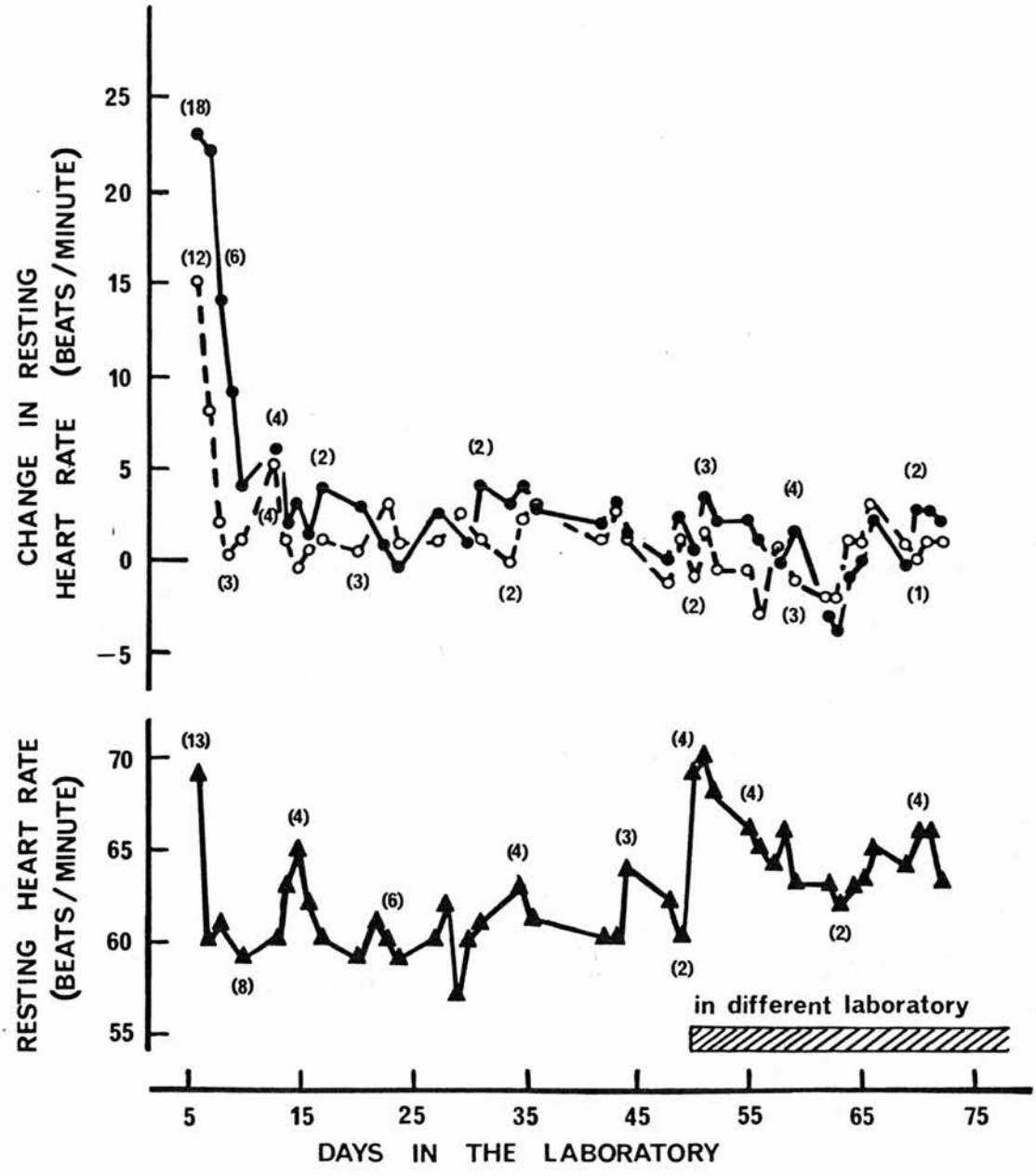

Figure 3:3

Changes in mean resting heart rate (HR_1, ▲) of four pregnant sheep during their first 72 days in the laboratory and changes in heart rate increment (HR_2-HR_1, ● ; HR_3-HR_1, ○) during handling over the same period (see text for details of HR_2 and HR_3). Standard deviations are given in brackets. The animals were moved to similar pens in another laboratory on day 50.

rise (HR_2-HR_1) decreased markedly before about day 10 and more slowly during the following 12-14 days. The heart rate after 5-7 min of handling (HR_3) was always less than HR_2 until day 20-22 and reached HR_1 values by about the sixteenth day. The consistent difference between HR_2-HR_1 and HR_3-HR_1 suggests that the animals became calmer despite, or as a result of, the continued presence of the experimenter. These four partially tamed ewes showed tame behaviour after 2-3 weeks of handling, when HR_2-HR_1 decreased to zero (Fig.3:3).

When the ewes were moved to similar pens in another laboratory on day 50, there was a significant ($P < 0.05$) increase in basal heart rate (HR_1) from 60 ± 2 on day 50 to 69 ± 5 beats/min on day 51, but the transient rise during handling did not return (HR_2-HR_1 and HR_3-HR_1 remained at zero).

3.4 DISCUSSION

This work (experiments 1 and 2) demonstrates that goats and sheep will usually adjust to a new laboratory environment within two weeks, unless they react adversely to a marked change in diet (Warner, 1962), and that sheep do not need to be handled for this process to be completed. Such animals will then be usable for experiments involving daily blood sampling. On the other hand, if animals are to remain unstressed by more prolonged experimental procedures 3-6 weeks of daily handling will be required. There was a close correlation between the disappearance of transient rises in plasma corticosteroid concentrations and heart rates and the appearance of tame behaviour, and thus the criteria of tameness used here seem to be satisfactory indices of the readiness of animals for

experiment.

Tamed goats were less disturbed than untamed goats when brought to the laboratory (experiment 1), and partially tamed sheep (experiment 4) took about three weeks to become ready for experiment while untamed sheep brought in directly from the field (experiment 3) took about six weeks. Therefore, the time taken for an animal to adapt seems to depend upon its previous experience of handling. This finding is in agreement with other studies (Reid and Mills, 1962; Bassett and Hinks, 1969; Purchas, 1973; Falconer, 1976) in which changes in plasma concentrations of glucose or corticosteroids were followed when sheep were moved to a strange environment, and during repeated venepuncture, restraint, or exposure to the sound of pistol shots or a barking dog. In every case the stress effects of the procedures were less in tamed animals or decreased as animals became accustomed to handling. But the time required for the stress effects to disappear was not examined. When daily handling was increased from about 10 min to 20-30 min, there was no apparent reduction in the time required for animals to become accustomed to prolonged procedures (Mellor, unpublished data).

In these goats (experiment 1) and in goats recovering from abdominal surgery (see Chapter Four) plasma glucose and corticosteroid concentrations were simultaneously elevated. The increased glucose concentrations may be an effect of the high corticosteroid levels, or an effect of adrenaline which, in sheep, can also cause an increase in plasma glucose concentrations (Setchell and McClymont, 1955). In contrast to these goat results, in sheep, relatively

small changes in plasma glucose levels were associated with transient increases in corticosteroid concentrations during repeated venepuncture (experiment 3) suggesting that the stress response in this particular study was different in goats and sheep during different procedures.

If the small rise in basal heart rate, which occurred when tamed animals were moved to an almost identical laboratory, was not associated with a change in stroke volume, it would have resulted in an increase in cardiac output of about 17%, that is a rise from about five to about six l/min. Whatever the magnitude of the change in cardiac output, the rise in heart rate shows that animals which have apparently adapted fully to experimental rooms and procedures still remain sensitive to even small changes in their conditions. So, if animals are to be moved from holding pens to a laboratory for regular physiological monitoring (Meschia et al., 1965; Strott, Sundel and Stahlman, 1974; Char and Creasy, 1976) this procedure should be included in any adaptation schedule.

3.5 CONCLUSIONS AND COMMENTS

1. Animals require 1-2 weeks to adjust to their new surroundings when brought into the laboratory. The period of adjustment is associated with depressed feed intakes and elevated plasma corticosteroid values and in some cases glucose concentrations.
2. Adaptation occurs whether or not animals are handled during the first 1-2 weeks.
3. The time required for animals to adapt to the laboratory and to experimental procedures requiring prolonged and direct contact

with the experimenter appears to depend upon their previous experience of handling.

4. From 3-6 weeks of handling are generally needed for animals to adapt to prolonged experimental procedures.
5. The close correspondence of behaviour and the physiological parameters makes behaviour a satisfactory index of the readiness of animals for experiment under the conditions in this laboratory. Before such an approach is adopted in different breeds or species or in different laboratories, similar parallels of behaviour and physiological parameters should be observed.
6. The sensitivity of the response of heart rate to 'emotional' stress and the immediacy of the measurement makes this a more useful parameter than plasma corticosteroid concentrations.
7. This study had design deficiencies as a result of the availability and subsequent requirements of animals. The deficiencies included the facts that (a) the same procedures were not conducted in both species and (b) the heart rate and corticosteroid measurements were not made in the same animals.
8. Animals that have apparently adapted fully remain sensitive to even small changes in their conditions. Therefore, experimental procedures should be included in any adaptation schedule.

CHAPTER FOUR

SOME PHYSIOLOGICAL CHANGES IN PREGNANT SHEEP AND GOATS BEFORE, DURING AND AFTER SURGICAL INSERTION OF UTERINE CATHETERS

4.1 INTRODUCTION

In spite of the relative advantages of the recovered, chronic preparation over the acute method in avoiding the presumed stress of anaesthesia and surgery, acute experiments are still carried out (e.g. Alexander, Britton, Forsling, Nixon and Ratcliffe, 1974a; Kiser, Convey, Lin and Oxender, 1975; Jones and Rurak, 1976), often because they provide the easy or only way of studying a particular problem or because animal holding facilities are limited. Although blood gas tensions, haematocrit and pH in acute preparations may be maintained at levels similar to those found in conscious, catheterised animals (Comline and Silver, 1970) these parameters give little idea of the stressful nature of the acute procedures. Marked differences have been found between fetuses examined under acute and chronic conditions. Perhaps the most striking was the discovery in goats and sheep that the progressive fetal hypoxia found in acute preparations as pregnancy advanced did not occur in conscious, unstressed animals (Meschia et al., 1965; Comline and Silver, 1970). However, there is apparently no direct evidence such as elevated plasma corticosteroid concentrations during operation, to indicate that acute procedures stress the mother. In addition, there is no agreement on the time required for mother and fetus to recover after an operation to insert uterine catheters. Dixon, Hyman, Gurpide, Dyrenfurth, Cohen, Bowe, Engel Daniel, James and Vande Wiele (1970) and Setchell, Bassett, Hinks and Graham (1972) report recovery after

2-3 hr when ewes had regained their feet and were eating. Shelley (1973), on the other hand, suggests 3-5 days may be required for sheep to recover from the effects of surgery after catheterisation.

In view of the lack of information on the stressful effects of acute procedures, the discrepancies in estimates of recovery time in sheep, and since there is no information for goats, some physiological changes in sheep and goats have been measured before, during and after insertion of uterine catheters.

4.2 EXPERIMENTAL METHODS

4.2.1 Animals

Eight sheep and four goats were operated on at 89-114 days and 81-98 days gestational age respectively, and 1-3 catheters were inserted into the conceptus.

4.2.2 Measurements

Maternal jugular blood was sampled each day before feeding or at 0900 hr on the two days preceding surgery, except in five animals when sampling was more frequent on the day of operation. In these five animals (four ewes and one goat) blood was sampled at the end of each stage of the operation (A-H). The time of sampling measured from when the animal entered the surgery has been shown in brackets in the following description. The course of operation was the same in all animals in this study.

Stages. A - blood sampled in the animal house (0.0 hr); B - a jugular vein catheterised under local anaesthetic in the surgery (0.1 hr); C - anaesthesia induced, endotracheal intubation and the

animal placed on the operating table (0.3 hr); D - the animal connected to pure oxygen, its abdomen prepared and a skin incision made (0.9 hr); E - exposure of the uterus, catheterisation and the uterus returned to the abdomen (1.7 hr); F - the peritoneum, muscle layers and skin sutured (2.3 hr); G - the endotracheal tube removed and the animal placed in a warm pen (3.2 hr); H - the animal conscious and attempting to stand (5.3 hr). At intervals during the operation the intravaginal temperature of each animal was taken.

Anaesthesia was induced with a mean of 24 mg pentobarbitone sodium/kg body wt in the sheep or 18 mg/kg in the goats and during the following 1.7-2.0 hr it was maintained with a further 23 mg/kg in sheep or 21 mg/kg in goats.

Results are expressed as mean $\pm$SD.

4.3 RESULTS AND DISCUSSION

4.3.1 Changes during operation

In four sheep and one goat (Fig.4:1A) plasma corticosteroid concentrations increased throughout the operation reaching about 100 ng/ml when final suturing was complete. Cessation of surgical stimulation resulted in a transient fall to about 70 ng/ml followed by a rise to about 120 ng/ml when the animals were conscious. Although no attempt was made to separate the effects of anaesthesia and surgery these results suggest that a major cause of the rise in corticosteroid concentrations was surgical trauma.

In all five animals intravaginal temperatures decreased during

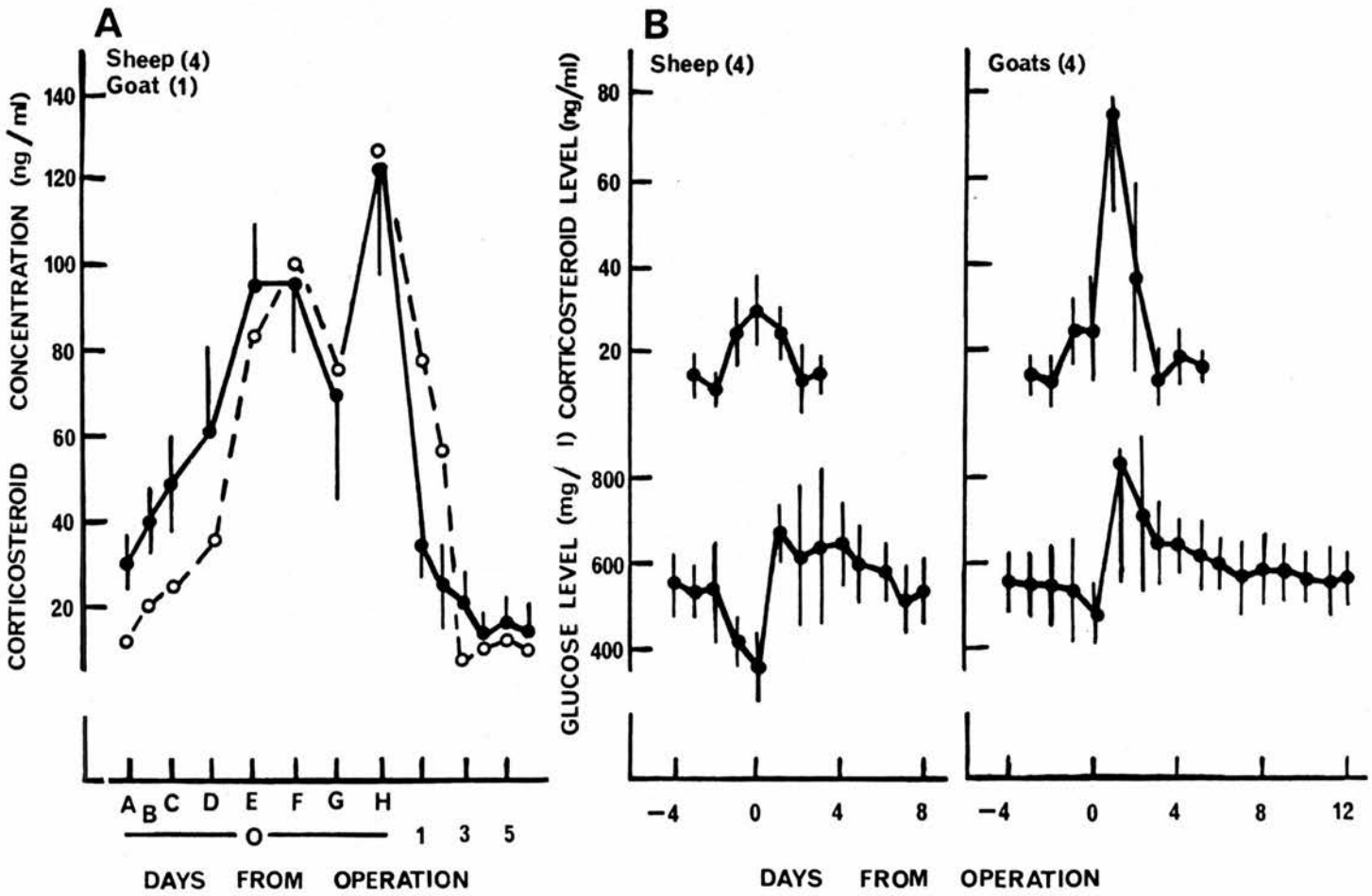

Figure 4:1

A. Changes in plasma corticosteroid concentrations during surgery (see text for description of stages A - H) and the subsequent six days in four sheep (mean ± SD) and one goat.

B. Daily changes in mean (± SD) plasma corticosteroid and glucose concentrations in four sheep and four goats around surgery.

the operation fmom 38-39°C to 35-36°C, the largest fall occurring when sterilising solutions were applied to the abdomen. Temperatures returned to preoperative values 6.0-6.5 hr after the start of operation.

Glucose concentrations (mean ($\pm$SD) - stage A = 440$\pm$80 mg/l (4) in sheep, 570 mg/l in one goat) remained relatively constant during operation in each animal, but there was considerable variation between animals. Mean values for each of the four sheep and the one goat during stages B-H were 650$\pm$50, 360$\pm$30, 570$\pm$50, 400$\pm$30 and 690$\pm$50 mg/l (n = 7), respectively.

No marked changes in plasma osmolality or Na concentrations were observed in the four sheep during operation but plasma K concentrations decreased significantly ($P < 0.05$) during the first hour (stages A-D). The lower concentrations were maintained until the animals regained consciousness and within 24 hr of operation had returned to preoperative values (Fig.4:2A). In one goat a similar but less marked decrease in K concentrations during operation was accompanied by an increase in plasma osmolality (Fig.4:2A)..These early changes in electrolytes seem likely to be due to presurgical procedures (starvation, restraint and induction of anaesthesia) rather than the surgery itself.

The hypothermia, high plasma corticosteroid concentrations and range of plasma glucose concentrations in anaesthetised, surgically manipulated animals complicate interpretation of results from acute experiments since most of them have been performed under similar

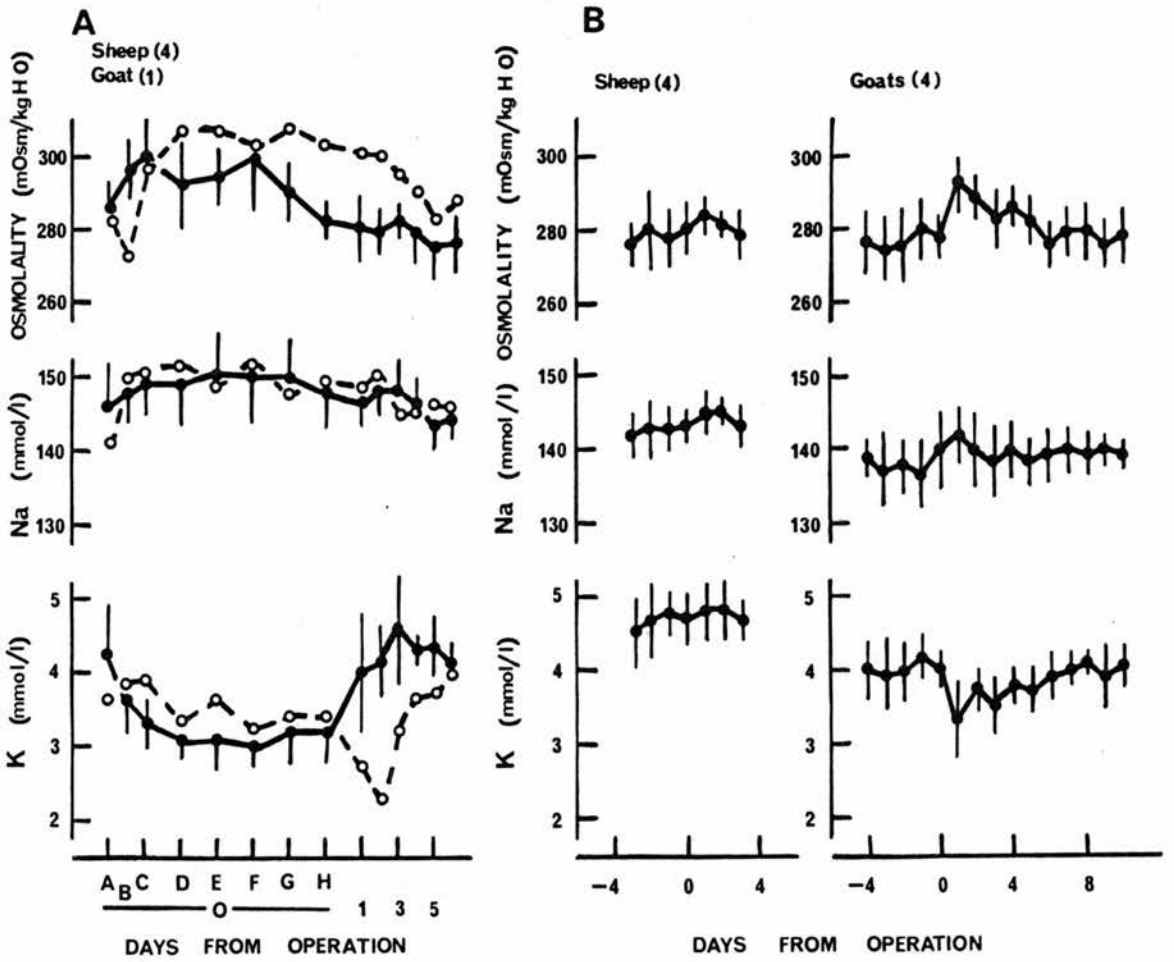

Figure 4:2

A. Changes in plasma osmolality and sodium and potassium concentrations during surgery (see text for description of stages A - H) and the subsequent six days in four sheep (mean (± SD) and one goat. B. Daily changes in mean (± SD) plasma osmolality and sodium and potassium concentrations in four sheep and four goats around surgery.

conditions (e.g. Dawes, 1968). These findings highlight the difficulty of relating observations in acute preparations to the situation in the conscious unstressed animal.

4.3.2 Changes before and after operation

In another four sheep and in four goats (Fig.4:1B) plasma corticosteroid concentrations increased and glucose values decreased during the 48 hr preoperative fast. This confirms similar observations in sheep (Purchas, 1973; Bassett and Madill, 1974a). Goats ate their straw bedding while feed was withheld which may partly explain why glucose levels were higher in goats than in sheep during fasting. In both species, feed intakes were usually between 10 and 50% of prefasting values until the second day after surgery. In sheep, intakes returned to prefasting values (i.e. 0.9 or 1.4 kg pelleted feed/day) on the third or fourth day after operation while in goats the intakes reached prefasting levels (i.e. 1.0 kg pelleted feed plus 0.3-0.5 kg hay/day) after five to seven days.

In both groups of sheep, mean plasma glucose concentrations stayed higher than prefasting values until seven days after operation (e.g. Fig.4:1). However, mean corticosteroid concentrations remained significantly higher ($P < 0.01$) than baseline values (12-16 ng/ml) for only three days in one group (Fig.4:1A) and one day in the other group (Fig.4:1B). This suggests that stress during recovery was greater in the first group which was catheterised at fetal ages of 104-114 days, 15-20 days later in pregnancy than the second group. The wide range of corticosteroid concentrations found one day after surgery in the eight sheep (range 19-94 ng/ml) is similar to that

found by other workers within 24 hr of surgery (Bassett and Thorburn, 1969; Beitins, Kowarski, Shermeta, De Lemos and Migeon, 1970; Dixon et al., 1970).

In the four goats, corticosteroid and glucose concentrations on the first two days after surgery were greater than those in the sheep (Fig.4:1), and goat plasma glucose levels took 10-12 days to return to prefasting values, which suggests that postoperative stress was greater in the goats than in the similarly treated sheep. This agrees with the subjective assessment, based on the degree of muscular tremor and ease of movement, that goats were more adversely affected by surgery than the sheep.

Plasma osmolality was significantly ($P<0.05$) higher and K concentrations were significantly ($P<0.01$) lower than prefasting values during the first two days after operation (Fig.4:2B) in the goats. Prefasting values were reached 6-8 days after operation when preoperative feeding patterns had been reestablished. No similar changes were observed in the sheep (Fig.4:2B). Regurgitation of rumen contents (2-5l) occurred in goats but not sheep during the operation. The goats drank up to 6-7l of 0.45% saline during the first 24 hr after operation, but sheep did not drink more than 4l. So the marked postoperative changes in plasma osmolality and K concentrations in goats may reflect a readjustment of a more disturbed maternal fluid balance than occurred in sheep.

Data obtained from experiments done within 2-3 days of surgery

(Flint, Anderson, Patten and Turnbull, 1974) while corticosteroid concentrations are likely to be elevated (Fig.4:1) probably apply to stressed animals with disturbed feeding patterns and fluid balance. Statements that animals had 'recovered' 2-3 hr after surgery (Dixon et al., 1970; Setchell et al., 1972) are not supported by the results of the present work. Indeed, recovery of the mother seems to require at least seven days in sheep or 12 days in goats, and there is evidence that sheep and goat fetuses require up to 16 days for electrolyte and hexose stability to be reached postoperatively (Mellor and Slater, 1972a, 1973b; Chapter Five).

4.4 CONCLUSIONS AND COMMENTS

1. Assuming, under the present experimental conditions, that marked rises in plasma corticosteroid concentrations result from stress-induced stimulation of the adrenal cortex, it may be concluded that anaesthesia and preoperative preparation of the abdomen are stressful to the mother, as is direct surgical stimulation.
2. Anaesthesia and surgery were associated with hypothermia.
3. Since animals are both stressed and hypothermic following operation, time must be allowed for recovery. Sheep require at least seven days and goats at least 12 days to recover after operation as assessed by a return to prestarvation feed intake, and plasma composition.
4. Changes in plasma corticosteroid values after operation in sheep suggest that postoperative stress is greater in late pregnancy.
5. Assuming the same sensitivity of the hypothalamo-pituitary-adrenal axis, and similar metabolic clearance of corticosteroids in both goats and sheep, goats are more adversely affected by

surgical procedures than sheep. This finding is substantiated by the immediate postoperative behaviour of goats relative to sheep.

6. The findings reported here are limited to the procedures in common use in this laboratory. Extensions to this work could include:

(a) The observation of more goats during operation.

(b) The use of a variety of anaesthetics and possible use of analgesics.

(c) Differentiation of the effects of anaesthesia and surgery.

(d) The use of different preoperative and surgical procedures.

CHAPTER FIVE

CHANGES IN FETAL FLUID COMPOSITION IN GOATS DURING THE LAST 60 DAYS OF PREGNANCY AND SOME OBSERVATIONS ON POSTOPERATIVE ABORTION

This chapter has been divided into two parts for clarity although the results described in each section were observed in the same experimental study.

PART ONE

5.1 INTRODUCTION

When applied to ruminant species other than sheep, the success of chronic catheterisation techniques has been limited. In cattle, catheterisation is often associated with premature delivery and retained placenta (Comline, Silver, Nathanielsz and Hall, 1973) while postoperative abortion may occur in goats (Thorburn and Schneider, 1972; Currie, 1974). A high incidence of postoperative abortion was encountered following catheterisation of the goat conceptus in the present study despite employing a recommended method of progesterone supplementation (Thorburn, Nicol, Bassett, Shutt and Cox, 1972) and surgical techniques which were completely successful in the sheep (Mellor, 1970a; Mellor et al., 1972). In attempts to prevent abortion, the progesterone supplement was varied from none to daily administration throughout pregnancy. The results are reported.

Although the endocrinology of the oestrous cycle, pregnancy and parturition, as reflected by changes in plasma hormone levels, has been examined in the goat (e.g. Bryant, Greenwood and Linzell,

1968; Linzell and Heap, 1968; Blom and Lyngset, 1971; Challis and Linzell, 1971, 1973; Irving, Jones and Knifton, 1972; Currie, 1974; Umo, Fitzpatrick and Ward, 1976) information on other plasma constituents is lacking. Therefore, plasma composition was examined in four uncatheterised goats during the oestrous cycle, pregnancy and parturition. This also allowed the composition of plasma from catheterised and uncatheterised goats to be compared.

5.2 EXPERIMENTAL METHODS

5.2.1 Animals

One pseudopregnant and 29 pregnant goats were used; 16 carried twins, 12 had single fetuses and one triplets.

5.2.2 Catheters

Between 81 and 111 days of gestation catheters were inserted into one or both fluid sacs of 36 fetuses in 25 goats and into the bladders of three of these fetuses. One goat, described as pseudopregnant, was mated 99 days before operation. A catheter was inserted into the body of the uterus which contained a fluid filled sac, but no cotyledons, placental vessels or fetus.

5.2.3 Progesterone treatment

Progesterone in oil (Organon Laboratories Ltd.) was given parentally once daily before feeding to most animals before and after operation in doses which were varied in attempts to prevent abortion. The initial treatment was a modification of that used by Thorburn *et al.* (1972). The first seven goats (group A) received 20 mg progesterone the day before, at operation (day 0) and for three

days thereafter, decreasing on day 4 to 10 mg, on day 5 to 5 mg and on day 6 to zero. The next three goats (group B) received 20 mg progesterone as before until days 4 or 5 when 10 mg were given and thereafter the dose was decreased by 1.25 mg per day to zero on days 12 or 13. Progesterone treatment was abandoned in the next 11 goats (group C), and in the pseudopregnant goat. The remaining four goats (group D) were given 10-15 mg progesterone per day from the day of operation until term.

5.2.4 Samples and analyses

Maternal jugular plasma was sampled daily until term or abortion in all operated goats (groups A to D) and throughout one oestrous cycle, pregnancy and parturition in four goats that were not catheterised (group E).

Plasma progesterone concentrations in the 25 operated goats were measured in samples taken from three days before operation until abortion or 13 days after operation. Samples were also analysed from nine operated goats (five and four from groups C and D, respectively) at 4-5 day intervals until 10 days before birth when daily changes were examined. Plasma progesterone concentrations were measured in samples taken from the four uncatheterised goats (group E) daily during one oestrous cycle and the first and last 10 days of pregnancy, and at four day intervals during the rest of pregnancy. Plasma LH concentrations were measured on a daily basis in these four goats.

Where appropriate, results are expressed as mean $\pm$SD.

5.3 RESULTS

5.3.1 Incidence of abortion

Abortion occurred in five of seven goats in group A 8-9 days after surgery, in one of three goats in group B after 13 days, and in the last four of 11 animals in group C after 2-3 days. None of the four goats in group D aborted. Although short-term progesterone treatment appeared to delay abortion in group A + B, the frequencies of abortion in group A + B and group C were not significantly different (X^2 test). All aborted fetuses were alive at delivery. The body weights of six goats that aborted and eight that did not were similar (range 35-78kg). The pregnancies of three catheterised goats were terminated 3-4 weeks after operation because of infection in the fluid sacs. Only twelve catheterised goats and the four unoperated goats carried their fetuses to term (148-155 days). Birth was normal and all kids were born alive but three (from three sets of catheterised twins) failed to breathe. Birth weights (2.2-3.3 kg) and growth rates during the first five weeks (0.9-1.6 kg/week) in the eighteen surviving kids from the catheterised goats and in the five kids from the uncatheterised goats were similar.

5.3.2 Plasma progesterone concentrations

When effects of twinning were eliminated in a co-variance analysis plasma progesterone concentrations in progesterone treated and untreated goats were not significantly different during the three days before operation and on the day of operation and showed no significant association with the subsequent abortion record. However, plasma progesterone concentrations measured in one goat that did not receive exogenous progesterone increased from

6.8±0.5 ng/ml (4) before to 10.5±1 ng/ml (4) during operation and had decreased to 5.8 ng/ml by the following day. This is consistent with other reports of increases in plasma progesterone concentrations in goats during anaesthesia and surgery (Heap and Linzell, 1966; Thorburn and Schneider, 1972). During the two days before abortion in the 10 goats plasma progesterone concentrations decreased from 7.6±4.2 to 0.8±0.5 ng/ml.

From 90 days of pregnancy (in unoperated goats) or from operation until 3-5 days before birth, progesterone concentrations were relatively constant and were generally higher in twin-bearing (7.6±3.0 ng/ml (151)) than in single-bearing (6.2±2.9 ng/ml (200)) goats. This is consistent with the findings of other workers (Irving et al., 1972; Thorburn and Schneider, 1972). In operated animals there was no significant difference between plasma concentrations in untreated (group C) goats and progesterone treated (group D) goats which were sampled 24 hours after progesterone injection. After allowing for effects of twinning progesterone concentrations were significantly lower ($P < 0.05$) in operated (group C) than in unoperated (group E) goats (corrected means 5.2 and 8.2 ng/ml, respectively).

During the last 3-5 days before birth in the nine untreated goats (groups C and E) progesterone concentrations decreased from 5-10 ng/ml to less than 1.5 ng/ml. But in the four progesterone treated goats (group D) birth occurred at 150-153 days of pregnancy despite the daily injections which maintained plasma progesterone concentrations of at least 4-7 ng/ml.

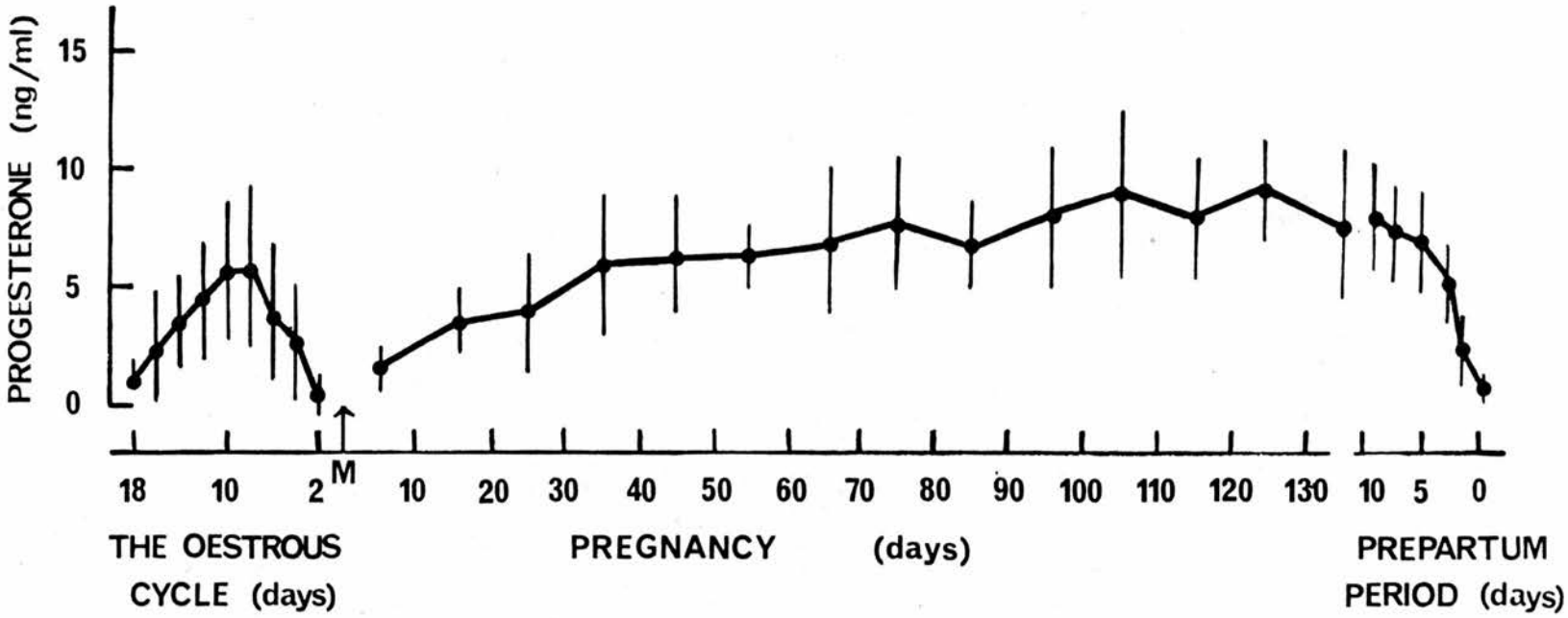

Figure 5:1

Changes in the progesterone concentration of plasma (mean (± SD) from three single-bearing goats and one twin-bearing goat during the oestrous cycle (two-day interval means) and pregnancy (ten-day interval means), including the prepartum period (two-day interval means). M = day of mating.

Blood samples were taken 24 hr after progesterone injection. Since Currie (1974) has shown that plasma progesterone concentrations increase and then decrease during the 24 hr after injection by the present method, these concentrations are likely to represent the minimum present during any 24 hr period.

5.3.3 Uncatheterised goats (group E)

As no teaser goat was available the start of the oestrous cycle (oestrus) in each goat was judged to be on the day when the lowest plasma progesterone concentration was observed 17-21 days before mating. During the oestrous cycle progesterone concentrations increased to 8±4 ng/ml 8-12 days after presumed oestrus and then decreased to low values (0.5±0.3 ng/ml (4)) on the day of mating. Maximum progesterone concentrations 8-12 days after oestrus were higher than those observed a similar time after mating in each of these four goats, although the group results was not significantly different. After mating the concentrations of progesterone, which were within the range observed by other workers (Irving et al., 1972; Thorburn and Schneider, 1972), increased during the first 70-90 days and then remained relatively constant until a prepartum decrease during the last 3-5 days of pregnancy (Fig.5:1). Plasma LH concentrations were low (0.81±0.37 ng/ml (43)) throughout the oestrous cycle, except for a marked rise within the 24 hrs before mating. Maximum concentrations observed were 8.2, 14.5, 56.0 and 70.5 ng/ml. Thereafter LH concentrations were generally low, being 1.39±1.08 ng/ml (80) during the first 20 days and 0.56±0.4 ng/ml (80) during the last 20 days of pregnancy. Low LH levels have also been observed during pregnancy in sheep (Niswender, Roche, Foster and Midgley, 1968; Alexander, Britton, Corker, Naftolin and Nixon, 1973a;

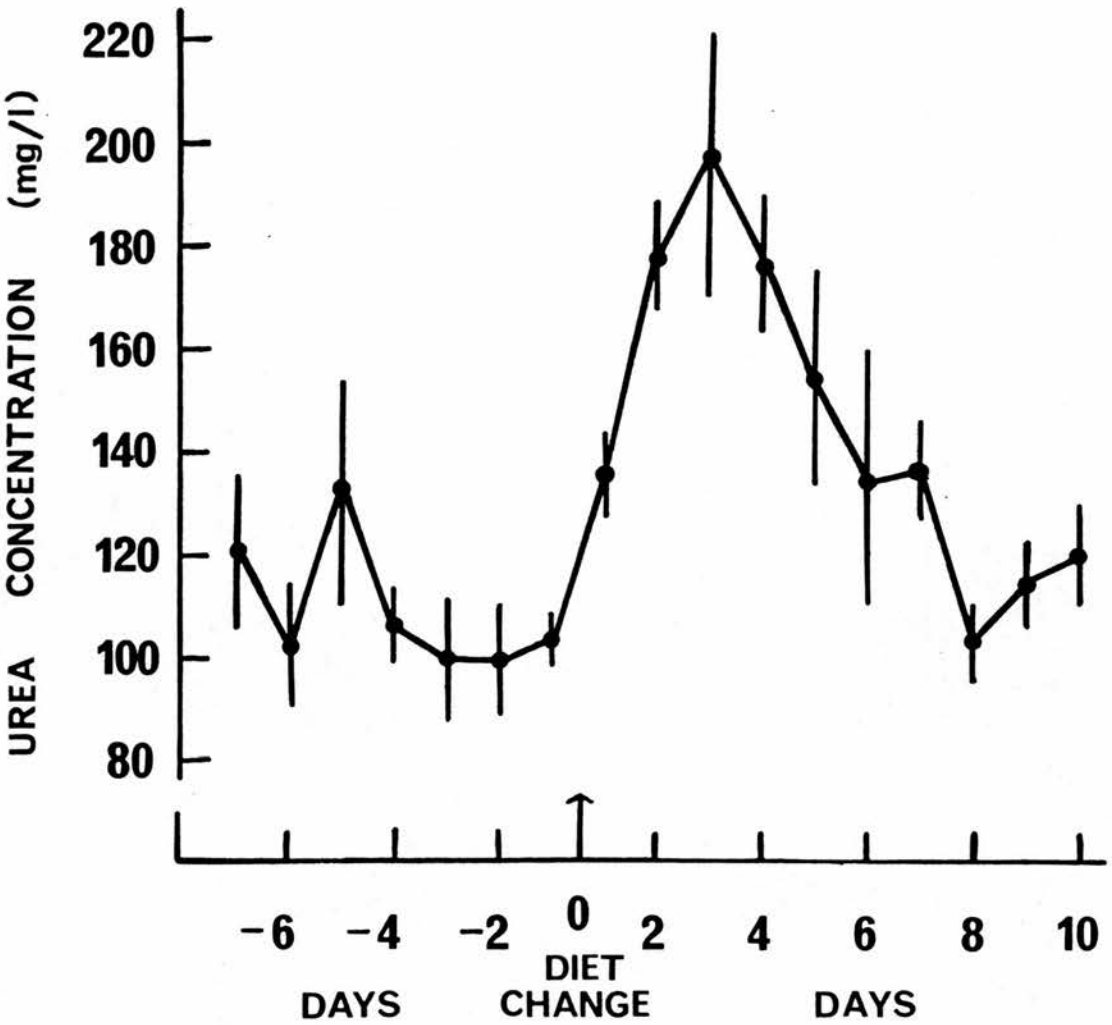

Figure 5:2

Changes in mean (± SE) plasma concentrations of urea in four uncatheterised goats when their diet was changed from one pelleted feed (Ewebol pencils) to another (Ruminant A) before 35 days of gestation.

Shevah, Black, Carr and Land, 1975) and cattle (Wettemann and Hafs, 1973; Arije, Wiltbank and Hopwood, 1974; Symons, 1976). Day to day variation in LH concentrations, which was considerable, showed a general tendency to decrease in each goat as pregnancy progressed as shown by the above SD. Plasma Na (140 ± 1.2 mmol/l (1264), K (4.1 ± 0.3 mmol/l (1264)), Ca 112 ± 4 mg/l (1254) and Mg (23.9 ± 1.6 mg/l (1254)) concentrations remained constant throughout the oestrous cycle and pregnancy and as in sheep (Scott and Robinson, 1976) remained relatively unchanged during parturition. Plasma P concentrations decreased during pregnancy from 59.2 ± 10.4 mg/l (66) during the oestrous cycle to 44.7 ± 9.0 mg/l (61) during the 10 days before parturition. Over the same period plasma urea concentrations increased from 134 ± 34 mg/l (60) to 186 ± 28 mg/l (42). Superimposed on these changes in plasma urea was a significant ($P<0.001$) but transient increase for 3 days in its concentration which occurred in each goat when the diet was changed from one pelleted feed (Ewebol pencils) to another (Ruminant A) at 18, 18, 33 or 34 days of pregnancy (Fig.5:2). At the same time a transient but less marked increase in plasma P concentration occurred but no related changes in plasma Na, K, Ca or Mg concentrations were observed.

5.4 DISCUSSION

A feature of this work was the high incidence (40%) of post-operative abortion. Abortion has been encountered in goats by other workers (Thorburn et al., 1972; Currie, 1974) but the frequency was not reported. Abortion was probably due to regression of the corpus luteum at or soon after surgery since abortion in goats given no progesterone (group C) was preceded by a decrease in plasma

progesterone concentrations which began within 24 hr of surgery. Luteolysis may have resulted from increases in prostaglandin $F_2\alpha$ ($PGF_2\alpha$) concentrations in uterine venous blood, which have been observed 3 hr after uterine surgery (Currie, 1974), as exogenous $PGF_2\alpha$ appears to be luteolytic in pregnant goats (Currie and Thorburn, 1973). Alternatively, luteolysis may have been induced by some effect of high plasma corticosteroid concentrations which are present during and immediately after operation (Chapter Four). The precise reasons for the occurence of abortion in some goats and not others are unknown. However, it is possible that the operation caused a reduction in progesterone secretion which in 40% of the goats reached levels which were insufficient to maintain pregnancy. This is suggested by the generally lower progesterone levels in non-aborting operated goats (group C) than unoperated goats (group E) and by the apparent reduction in ovarian production rate of progesterone in goats during anaesthesia and surgery (Linzell and Heap, 1968; Heap, Bedford and Linzell, 1975a). However, the differences between plasma progesterone levels in group C and E goats may equally have been due to utilisation or secretory differences. A decrease in the utilisation of progesterone, possibly associated with a change in blood flow through progesterone metabolising tissues, and an increase in the production rate of progesterone by the adrenal glands during operation would perhaps explain the increase in plasma progesterone concentrations during anaesthesia and surgery in goats (the present study; Heap and Linzell, 1966; Thorburn and Schneider, 1972). Clearly the nature of progesterone secretion and metabolism at operation warrants further study in view of the high incidence of

postoperative abortion in goats.

Exogenous progesterone did not decrease the incidence of luteolysis, but appeared to delay abortion in those goats in which the corpus luteum had regressed. The production rate of progesterone during pregnancy in Saanen goats is about 22 mg/day (Heap et al., 1975a) so, until withdrawal, the 10-20 mg/day given to the generally smaller goats in this study should have at least partly replaced secretion by the corpus luteum. Plasma progesterone levels in samples obtained 24 hr after progesterone injection appeared to be in the physiological range in the four goats given progesterone daily until term (group D). However, since plasma progesterone concentrations increase transiently after injection by the present method (Currie, 1974), these concentrations are likely to represent the lowest values for these goats during the day. It was not possible to establish whether luteolysis had occurred after surgery in these goats (group D), so it cannot be claimed that this treatment prevented abortion, but in these animals the plasma progesterone concentrations measured 24 hr after progesterone injection did not decrease before parturition.

Parturition in the goat is normally preceded by luteolysis and a decrease in plasma progesterone levels, due apparently to increased fetal adrenocortical activity (Thorburn et al., 1972; Currie, 1974). The findings here suggest that these changes may not all be necessary for birth to occur. Birth occurred at the normal time in the goats given progesterone daily (group D) despite the absence of a preparturient decrease in plasma progesterone

concentrations. A possible explanation is that the progesterone given these animals (10-15 mg/day) was not sufficient to overcome effects of the active participation of the goat fetus in the birth process. On the other hand, the marked decrease in plasma progesterone concentrations in the pseudopregnant goat before delivery (Section 5.7.3), shows that luteolysis at term can result from non-fetal factors. This suggestion is further supported by the less abrupt decrease in plasma progesterone concentrations which occurred 147 to 184 days after mating in six goats hysterectomised after pregnancy had been established (Currie and Thorburn, 1974).

The lower levels of progesterone observed in early pregnancy (at 8-12 days of gestation) compared with those found during the luteal phase of the oestrous cycle suggest that the corpus luteum of pregnancy is different from that of the cycle, or that progesterone metabolism is altered by some effect of the presence of the conceptus. Clearly, more goats would need to be examined before any significant difference could be established, particularly as the difference has not been observed by other workers (Heap and Linzell, 1966; Thorburn and Schneider, 1972).

Plasma progesterone concentrations during pregnancy will be determined by several factors. A placental luteotrophin, as suggested by Thorburn and Schneider (1972), or anti-luteolysin, may affect progesterone secretion, since an increase in progesterone concentration occurs during the first 70-80 days of pregnancy when placental development would be taking place (van Rensburg, 1971). Other factors such as the number of corpora lutea and fetuses and

the metabolic rate may also be involved. Furthermore, in pregnant sheep, plasma progesterone concentrations increase and are positively correlated with plasma free fatty acid (FFA) concentrations when ewes are undernourished (Shevah et al., 1975). In late pregnancy, goats with twins are likely to be undernourished relative to single-bearing goats, because of greater fetal requirements. So, the higher progesterone concentrations observed in twin-bearing relative to single-bearing goats in this and other studies (Irving et al., 1972; Thorburn and Schneider, 1972) may be at least partly due to an effect of nutrition.

Compared with the peak LH concentration observed before mating, LH levels during the rest of the oestrous cycle and pregnancy were low, and never increased above 3.5 ng/ml. Therefore, any effects of LH on ovarian function during pregnancy are likely to be small. Consistent with this suggestion is the lack of any relationship between LH and progesterone concentrations during pregnancy, although LH concentrations in the last third of pregnancy were generally low when progesterone concentrations were at their highest. No marked transient increase in the concentrations of LH like those found in mid-pregnancy in a cow (Carr, 1971) were observed during goat pregnancy, but with once daily sampling sudden, short lived increases in LH activity could have been missed. Since the time of sampling relative to the preovulatory peak in LH secretion would almost certainly have been different in the four animals it is not surprising that there was marked variability in the maximum LH concentration measured during the oestrous cycle.

PART TWO

5.5 INTRODUCTION

A relatively small number of experiments have been reported in conscious goats (e.g. Meschia et al., 1965; Thorburn et al., 1972; Currie, 1974) and cows (Comline, Hall, Lavelle, Nathanielsz and Silver, 1974; Peterson, Hunter, Welch and Fairclough, 1975; Comline and Silver, 1976) and they are mainly concerned with the investigation of changes at parturition. Therefore, although the fetal fluids of the sheep have been examined in detail in conscious, catheterised animals after about 60 days of pregnancy (Mellor and Slater, 1971, 1972a, 1973b), there is no comparable information for goats.

Species differences may be expected because of the higher production rates of progesterone in sheep than in goats after about 60 days of pregnancy (Bedford, Harrison and Heap, 1972; Heap et al., 1975a). In the goat, the corpus luteum remains the major site of progesterone production throughout pregnancy but in sheep after about 60 days and in cows after about 200 days the placenta becomes the major site of production (Estergreen, Frost, Gomes, Erb and Bullard, 1967; Linzell and Heap, 1968; Thorburn and Schneider, 1972; Bassett and Thorburn, 1973; Heap, Henville and Linzell, 1975b). McGovern (1976) and Alexander and Williams (1968) have shown that the volume and chemical composition of fetal fluids of ovariectomised goats and sheep are likely to be sensitive to concentrations of circulating progesterone. Progesterone produced in the sheep and possibly the cow placenta (Estergreen et al., 1967; Linzell and Heap, 1968) may by local action modify uterine blood flow (Caton, Abrams, Lackore, James and Barron, 1974) or alter the permeability of the

uterine blood vessels (Hawk, Brinsfield and Righter, 1963) or fetal membranes.

Changes in the composition of fetal fluids from conscious goats are now described and compared with similar sheep data (Mellor and Slater, 1971, 1973b; Mellor and Matheson, 1977) and with other information on the composition of fetal fluids from anaesthetised cows (Reeves et al., 1972) or cows after slaughter (Thomsen and Edelfors, 1976).

5.6 EXPERIMENTAL METHODS

These were as described in Section 5.2. Amniotic fluid, allantoic fluid, fetal urine and maternal jugular plasma were sampled daily until term or abortion in the catheterised animals.

5.7 RESULTS

5.7.1 Fetal fluid and maternal plasma composition

Postoperative changes in amniotic and allantoic fluid were qualitatively similar in each of the 12 fetuses whether abortion occurred or not and were similar to those reported for sheep (Mellor and Slater, 1971, 1973b; Mellor and Matheson, 1977). Mean values for the different substances are given in Table 5:1. The time required for the establishment of stability or of subsequently observed gestational trends depended upon the substance being considered. However, effects of operation on both fluids appeared to have passed after 7-10 days in all goats.

Plasma from 16 goats was examined after operation. It took

TABLE 5:1

Changes in the mean composition of amniotic fluid (AMF) and allantoic fluid (ALF) during the first 14 days after operation in a total of 12 goats that did not abort after operation (op). The average standard deviation ($\bar{X}$SD) is given for each parameter

		Days after operation										(n) No. obs per mean
		op	1	2	3	4	5	7	10	14	$\bar{X}$SD	
Osmol. mOsm/kg H_2O	AMF	258	358	308	310	306	293	298	296	302	34	9-10
	ALF	269	317	337	325	305	300	315	334	326	28	7-10
Na mmol/l	AMF	123	128	126	124	123	129	130	128	124	7.0	9-10
	ALF	20	27	36	43	48	53	53	55	69	22	7-10
K mmol/l	AMF	7.2	7.8	7.0	5.9	5.0	5.0	5.2	5.7	6.1	1.0	9-10
	ALF	63	54	49	46	43	42	43	50	48	26	7-10
Cl mmol/l	AMF	113	126	123	116	118	112	119	123	125	11	8-10
	ALF	19	23	30	31	34	36	43	44	58	21	7-10
Urea mg/l	AMF	210	240	280	260	190	170	170	160	160	48	9-10
	ALF	380	390	400	430	370	320	270	250	220	99	7-10
Fructose mg/l	AMF	1360	1640	1610	1460	1650	1550	1830	2450	2750	630	8-10
	ALF	1790	1930	2700	2780	3350	3820	3870	2680	2390	1370	6-8
Total Ca mg/l	AMF	134	360	510	290	134	180	155	108	120	47	3-4
	ALF	700	850	820	710	550	530	460	540	490	270	6-8
Total Mg mg/l	AMF	9.7	13.0	19.5	14.5	10.0	12.0	12.0	10.0	17.0	5.0	3-4
	ALF	90	84	69	80	82	84	94	97	88	23.0	6-8

from 5 to 12 days for maternal plasma urea, glucose and K concentrations to return to prefasting levels. There was a significant ($P < 0.001$) decrease in urea from 310 ± 90 (day 1) to 170 ± 40 mg/l (day 5) and glucose from 870 ± 190 (day 1) to 590 ± 60 mg/l (day 12) and a significant ($P < 0.001$) increase in K from 3.4 ± 0.3 (day 1) to 3.9 ± 0.3 mmol/l (day 7) after operation. These observations extend those reported in Chapter Four.

Gestational changes. During the last 70 days of pregnancy changes in the composition of amniotic fluid were distinct and were qualitatively similar in each of six fetuses (Table 5:2). Few samples were obtained after 140 days because amniotic fluid became increasingly gelatinous and blocked most catheters.

Allantoic fluid was sampled for 20-45 days in seven fetuses. Few samples were obtained after 135 days. Osmolality (330 ± 32 mOsm/kg water (110)) was similar in each fetus, and showed day to day fluctuations of 10-40 mOsm/kg water with a general tendency to increase during the sampling period. Na and K concentrations showed large variations both between and within each goat (e.g. Fig.5:3). Within each fetus there was a significant ($P < 0.01$) negative correlation between the Na and K concentrations in allantoic fluid ($r = -0.50$ to -0.98, $n = 21-42$) as in the sheep. However, unlike the sheep (Mellor and Slater, 1972a) between-animal variations in Na and K concentrations were not related to differences in maternal plasma glucose concentrations. Cl concentrations were 20-45 mmol/l until about 118 days of gestation when concentrations increased continuously to a maximum of 87 ± 7

mmol/l (7) which was reached between 125 and 135 days (e.g. Fig.5:3). Allantoic fluid fructose concentrations showed linear decreases in each fetus. Highest values were 1800-6500 mg/l and lowest were 900-3400 mg/l. The urea concentrations remained relatively constant in fluid from each animal (concentration range 200-380 mg/l). Mg concentrations remained relatively constant and were similar in each fetus (range 82-104 mg/l), but Ca concentrations decreased from 520-1600 mg/l during the first 2-3 days after operation, to 100-450 mg/l after 20-45 days. The compositions of fluid from each end of the allantoic sac of one fetus were similar and showed the same trends.

After recovery, the concentrations of all substances in maternal plasma remained relatively constant in operated goats that carried their fetuses to term (e.g. Table 5:2) and were similar to those observed in unoperated goats (section 5.3.3).

5.7.2 Composition of fetal urine

Daily samples were obtained from three fetuses until abortion 8, 9 and 13 days after operation. The results for each parameter between the first and seventh day after operation were pooled. Mean $\pm$SD (n) values were: osmolality 347$\pm$102 mOsm/kg water (20), Na 59$\pm$11 mmol/l (21), K 6.9$\pm$2.3 mmol/l (21), Cl 43$\pm$12 mmol/l (21), fructose 2570$\pm$1690 mg/l (21) and urea 580$\pm$250 mg/l (21).

5.7.3 The pseudopregnant goat

This goat showed all the characteristics of 'cloudburst' reported in domestic goats (Mackenzie, 1970; Miller and West, 1970).

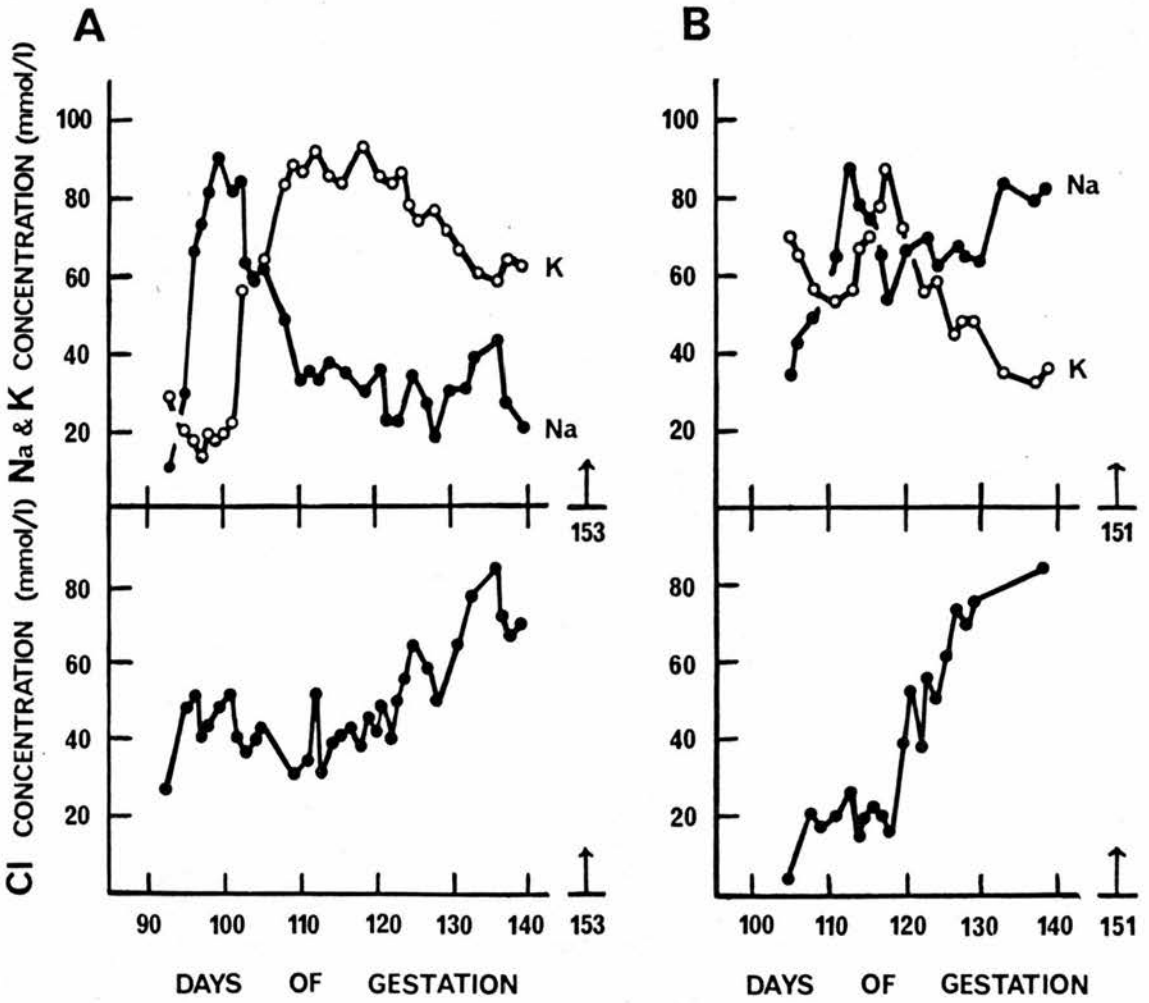

Figure 5:3

Gestational changes in the sodium, potassium and chloride concentrations in allantoic fluid from one triplet (A) and one twin fetus (B) in two goats catheterised at 93 (A) and 105 (B) days of gestation. Birth occurred at 153 and 151 days of gestation as indicated.

Following operation fluid samples were obtained daily until 153 days after mating when the fluid and membranes were delivered following an apparently normal labour. Between 99 days and 150 days the fluid pH, osmolality and urea concentration were 5.7-6.2, 200-350 mOsm/kg water and 250-400 mg/l, respectively, and the Na concentrations decreased from 85 to 45 and Cl from 140 to 120 mmol/l. There was less than 0.5 mmol/l of K and no fructose in the fluid, while Ca and Mg concentrations increased from 550 to 700 and 45 to 65 mg/l, respectively. The marked difference between the fluid and plasma composition may have been due to membrane processes. The deficiency of fructose confirms that no placental tissue was present, since fructose appears to be largely produced in the placenta (Britton et al., 1967). During the three days before delivery plasma concentrations of progesterone in this goat decreased from 9 to 0.4 ng/ml and in the uterine fluid there were continuous decreases in the concentrations of Cl (from 120 to 83 mmol/l) Ca (from 700 to 520 mg/l) and Mg (from 65 to 45 mg/l) and continuous increases in Na (from 45 to 70 mmol/l) and K (from 0.5 to 4.5 mmol/l) concentrations.

Uterine fluid accumulation has also been observed under different circumstances in sheep. Harrison, Heap and Poyser (1976) found that fluid accumulated in the uterus of a non-pregnant ewe given 10 or 20 mg progesterone each day for 115 days. In this case no membranes were apparent and the fluid was of a different composition to that found in the present goat although similar to fluid obtained from surgically prepared uterine pouches in pregnant ewes from which the conceptus was excluded (Harrison et al., 1976).

5.8 DISCUSSION

Postoperative changes in the fetal fluids were similar to those observed in sheep (Mellor and Slater, 1971, 1973b; Mellor and Matheson, 1977). In the sheep, the differences in fetal fluid hexose balance and the electrolyte composition of allantoic fluid at operation and during recovery appear to be due largely to effects on the ewe of a 48 hr preoperative fast. On the other hand, changes in fetal fluid osmolality and amniotic fluid K concentrations seem to be associated with the operation itself (Mellor and Slater, 1971). Because of the lack of successful goat preparations in the present study it was not possible to differentiate between effects on the fetal fluids of starvation, anaesthesia, surgery and other related factors. However, the similarity between the changes observed in goats and sheep after operation (e.g. the hypotonicity of the fluids at operation relative to the rest of gestation, the decrease in amniotic fluid K concentrations and the changes in allantoic fluid electrolyte, fructose and urea concentrations; Table 5:1; Mellor and Slater 1971, 1973b) suggest that similar factors may influence the composition of the fetal fluids in both species during the first 8-14 days after catheterisation.

The composition of amniotic fluid from goats (Table 5:2), sheep (Mellor and Slater, 1971, 1973b; Mellor and Matheson, 1977) and cattle (Reeves et al., 1972) are generally similar, so that progesterone produced from different sites in the three species apparently has little effect on amniotic fluid. The most striking similarity was the high Cl concentrations in amniotic fluid relative to maternal or fetal plasma in all three species. Mellor (1970b)

studied the electrical potential differences (p.d.s) between amniotic fluid and fetal plasma in five goats and 14 sheep, and found that until shortly before birth the Cl concentrations of amniotic fluid were greater than would be present at electrochemical equilibrium. He therefore concluded that Cl was actively transported into the amniotic sac. In all three species after about 85% of pregnancy has been completed (sheep, 124 days; goats, 130 days; cattle, 235 days) the Cl concentrations decrease to below maternal plasma values. This decrease in Cl concentration after about 124 days in sheep in Mellor's (1970b) study was associated with an increase towards zero of the p.d. between amniotic fluid and fetal plasma, consistent with a decrease in Cl pumping into the amniotic sac (Mellor, 1970b). The similarity between the amniotic fluid Cl changes in conscious goats (Table 5:2) and anaesthetised cows (Reeves et al., 1972) and those in anaesthetised (Mellor, 1970b) and conscious (Mellor and Slater, 1971) sheep, suggests that active transport of Cl into the amniotic sac during most of the last half of pregnancy is a feature of the ruminant conceptus.

Cl ions account for about one third of the osmolality of the amniotic fluid in goats (Table 5:2) and sheep (Mellor and Slater, 1971) before the last 15% of pregnancy. In order to maintain electrochemical balance the same number of anions and cations must enter the fluid, so that Cl pumping into amniotic fluid would make a significant contribution to the number of osmotically active particles in the amniotic sac. Therefore, active transport of Cl is likely to be an important factor maintaining amniotic fluid volume before fetal urine enters the sac in detectable quantities.

In sheep after 80 days (Alexander, Nixon, Widdas and Wohlzogen, 1958b; Mellor and Slater, 1971, 1972a) and in cows after 230 days of gestation (Reeves et al., 1972) some of the changes in electrolyte, fructose and urea concentrations in amniotic fluid have been attributed to an increasing flow of fetal urine into the amniotic sac. Since caprine (present study) and ovine (Mellor and Slater, 1972a) fetal urine have similar compositions, urine flow may also account for the similar changes in amniotic fluid composition observed in the goats (Table 5:2). For example, such a flow would account for the changes in the Na, K and urea concentrations of amniotic fluid, but would need to be combined with decreasing fructose concentrations in fetal urine to account for the decrease in the fluid fructose concentrations after 110 days of gestation. However, until direct measurements are made on the rate of urine flow into the amniotic and allantoic sacs, statements about alterations in the distribution of fetal urine flow between the two sacs and effects of urine on fetal fluid composition will remain unsubstantiated.

Amniotic fluid was hypertonic to maternal plasma in the goats (Table 5:2) but hypotonic during the same period in sheep. If fetal urine enters both fluid sacs in the goat as it may in the sheep (Alexander et al., 1958b; Mellor and Slater, 1972a), the generally higher osmolality of fetal urine in the goat could account for the hypertonicity of both amniotic and allantoic fluid in this species. However, the fetal urine data from the present goats was obtained within eight days of surgery and should be viewed with caution because of lingering effects of operative procedures.

Changes in allantoic fluid composition until 135 days in the goats were generally similar to those observed in sheep. As in sheep (Mellor and Slater, 1971) an inverse relationship was observed between the Na and K concentrations of allantoic fluid, but the pattern of change in Na and K concentrations was markedly different in each goat (e.g. Fig.5:3) despite the fact that they received similar treatment. This contrasts with sheep which, when exposed to similar nutritional and husbandry procedures, show strikingly consistent electrolyte patterns in allantoic fluid (Mellor and Slater, 1971). Higher Cl concentrations (60-90 mmol/l) were found in allantoic fluid from the goats after about 118 days of gestation than are normally present in fluid from conscious sheep (usually $<$ 50 mmol/l: Mellor and Slater, 1971). In addition, the Cl concentrations of allantoic fluid from anaesthetised goats, sheep and cows rarely exceed 35 mmol/l (Mellor, 1970b; Reeves et al., 1972).

Regulation of the Na and K concentrations of ovine allantoic fluid may be due to an action of fetal corticosteroids on pumping mechanisms in the chorioallantois (Mellor et al., 1975b). If this is true in the goat, the more erratic changes in the fluid Na and K concentrations in goats compared to similarly treated sheep could reflect a greater sensitivity of the goat chorioallantois to changes in corticosteroid concentrations, or a more responsive or less well regulated fetal adrenal than is found in sheep. On the other hand, this species difference could have been associated with progesterone. Although it was not possible, because of the small numbers, to differentiate between the effects of the various progesterone treatments, it is of interest that four of the fetuses showing the

most erratic electrolyte patterns (e.g. Fig.5:3B) received a progesterone supplement daily, and that the most consistent and regular changes in allantoic fluid occurred in a goat that received no progesterone treatment (Fig.5:3A). In addition, although the different progesterone treatments prevented an assessment of associations between species differences in progesterone production and differences in allantoic fluid composition, the apparent lack of much placental progesterone production in goats (Linzell and Heap, 1968) may have been another factor influencing fluid electrolyte composition.

The low Cl concentrations in allantoic fluid from anaesthetised sheep and goats (Mellor, 1970b) can be accounted for by the p.d.s between allantoic fluid and maternal and fetal plasma, because the negativity of the fluid relative to the plasma would promote diffusion of Cl ions out of the allantoic sac (Mellor, 1970b). These p.d.s are thought to be generated, at least partly, by active transport of Na out of allantoic fluid. Therefore, the marked rise in Cl concentrations of allantoic fluid in conscious goats (e.g. Fig.5:1) to values approaching those found in plasma can be accounted for by one or both of two factors: (1) an increase in the permeability of the chorioallantois, which seems unlikely as no simultaneous marked changes in hexose and urea concentrations occurred, or (2) a decrease in the Na pumping activity which would reduce the negativity of the allantoic sac with respect to maternal and fetal plasma and would allow an influx of Cl ions into the sac. Changes in allantoic fluid Na and K concentrations at the time Cl levels increased are consistent with a decrease in pumping activity

across the membrane.

In the sheep, fructose is formed from glucose in the placenta (Britton et al., 1967), is excreted by the fetal kidneys (Mellor and Slater, 1972a) and in fetal urine appears to enter both fluid sacs (Mellor and Slater, 1973b). After birth it disappears from the blood of the lamb within 1-2 days (Cole and Hitchcock, 1946; Shelley, 1960; Chapter Eight). Fructose concentrations in amniotic fluid, allantoic fluid and fetal urine in goats (present study) and cattle (Reeves et al., 1972) are similar to those observed in sheep, and a rapid disappearance of fructose from plasma also occurs after birth in the newborn kid (Chapter Eight) and calf (Edwards, 1970). Thus, the sites of production and the distribution of fructose in the conceptuses of these three species are likely to be similar, but some differences are apparent. In the present goats, allantoic fluid fructose concentrations decreased steadily while maternal plasma glucose concentrations remained relatively constant, in contrast to the sheep in which maternal plasma glucose and allantoic fluid fructose concentrations change in parallel (Mellor and Slater, 1973b). If the net rate of entry of fructose into the allantoic sac remains constant then the decrease in fructose concentration in allantoic fluid in the goats will reflect an increase in fluid volume in the sac. Allantoic fluid appears to increase in volume in late gestation in sheep and cattle (Malan, Malan and Curson, 1937; Cloete, 1939; Klenov, 1973). However, difficulties in obtaining allantoic fluid from similar catheters in sheep was usually associated with a reduction in allantoic fluid volume (Mellor, unpublished data). If this is true of the present goats then the volume may have been

fairly constant or decreased with increasing gestational age. The decrease in fructose would then be due to a reduction in the net entry rate of fructose into the sac as a result of one or all of the following: (1) a decrease in the flow of fetal urine into the allantoic sac in the last third of pregnancy similar to that which may occur in cattle (Reeves et al., 1972) but not in sheep (Mellor and Slater, 1973b), (2) a decrease in placental synthesis of fructose, or (3) an increase in the rate of loss of fructose from the allantoic fluid. However, in the absence of direct volume measurements the reasons for the gestational decrease in allantoic fluid fructose remain unknown. Between-goat differences in maternal plasma glucose concentrations were small but between-fetus differences in fructose concentrations of both amniotic and allantoic fluid were large although similar trends were observed in each fetus. This suggests either large differences in allantoic fluid volume between fetuses which seems unlikely (van Rensburg, 1971; McGovern, 1976), or large differences in placental fructose synthesis or urinary excretion between fetuses.

The greater concentrations of Ca and Mg in allantoic fluid relative to maternal plasma in the goat are of interest. Assuming that, as in sheep (Bawden, Wolkoff and Flowers, 1965; Delivoria-Papadopoulos, Battaglia, Bruns and Meschia, 1967; Suttle and Field, 1969) about 55-65% of Ca and Mg in plasma is not bound to protein and that all Ca and Mg in allantoic fluid is ionic, with a p.d. between fetal plasma and allantoic fluid of -30 to -50 mV (Mellor, 1970b), fluid Ca and Mg concentrations of 610-2650 mg/l and 150-630 mg/l, respectively, could be maintained by the electro-

chemical gradient. Observed Ca and Mg concentrations were within these limits, so it is not necessary to postulate active transport, even if the chorioallantois as a whole is impermeable to Ca and Mg. If the whole membrane is permeable and Ca and Mg can pass between maternal plasma and allantoic fluid, then the electrochemical gradient between maternal plasma and allantoic fluid (-88 to -136 mV: Mellor, 1970b) is more than sufficient to account for the concentration of Ca and Mg in allantoic fluid.

5.9 CONCLUSIONS AND COMMENTS

1. Plasma progesterone concentrations in pregnant goats are readily disturbed by operative procedures.
2. There is a high incidence of postoperative abortion, apparently due to luteolysis, in goats unlike sheep. This is not reduced by exogenous progesterone supplementation (10-25 mg progesterone/day) although exogenous progesterone can delay abortion.
3. Daily injections of 10-15 mg progesterone are not sufficient to prevent parturition in goats at term weighing 50-60 kg.
4. Luteolysis at term in goats can result from non-fetal factors suggesting that a maternal stimulus to parturition may act in the absence of or reinforcing a fetal stimulus in the goat.
5. As in sheep, preoperative starvation, anaesthesia and surgery are likely to influence the composition of the fetal fluids from goats during the first 8-14 days after catheterisation.
6. The different sites of progesterone production in the sheep, goat and cow appear to have little effect upon amniotic fluid composition since its composition in conscious goats and sheep and anaesthetised cattle is similar.

7. Active transport of Cl ions into the amniotic sac during pregnancy may be a feature of the ruminant conceptus.

8. Fetal urine flowing into the amniotic sac may affect caprine amniotic fluid composition particularly in late pregnancy.

9. The small number of goats from which allantoic fluid samples were obtained and the considerable variation in fluid composition between goats made interpretation of results difficult. Allantoic fluid composition is generally similar to that observed in sheep but some differences are apparent.

   (a) Fluctuations in Na and K concentrations (which are inversely related) before 125 days in gestation are more marked in goats than sheep.

   (b) There is a marked increase in Cl concentration in caprine allantoic fluid at about 125 days which may be the result of a decrease in the Na pumping activity of the chorioallantois. Changes in Na and K concentrations are consistent with this.

10. The species differences in allantoic fluid composition may have been associated with the lack of marked placental progesterone production in the goat unlike the sheep, but it is not possible to distinguish between this effect and effects of a daily progesterone supplement, which was given to four of the seven goats.

11. Except for plasma progesterone concentrations, catheterisation apparently had no long lasting effects on plasma composition in goats.

12. Further work in this area to extend the present observations could include:

   (a) An investigation of the use of inhibitors of prostaglandin

synthesis to prevent abortion after surgery in the goat.

(b) A more detailed examination of the effect of exogenous progesterone supplementation on parturition at term and of operative procedures on plasma progesterone concentrations.

(c) Additional data on the composition of caprine allantoic fluid.

(d) An examination of the composition of caprine fetal urine after postoperative recovery to determine whether the relatively high osmolality of fetal urine is associated with the catheterisation or whether the hypertonicity of the fetal fluids of the goat relative to those of the sheep can be accounted for by a greater tonicity of fetal urine in goats.

CHAPTER SIX

THE COMPOSITION OF RUMINAL AND ABOMASAL FLUID FROM FETAL SHEEP DURING THE LAST 50 DAYS OF PREGNANCY

6.1 INTRODUCTION

Due largely to the success of the chronic sheep preparation, changes in sheep fetuses during late pregnancy are well documented (e.g. Comline and Silver, 1974). However, some areas (e.g. the fetal digestive tract) have as yet received little attention. To date the reticulo-rumen, omasum and abomasum have not been studied in the fetus in conscious animals, although, after birth their development and function have been extensively studied, particularly in cattle (e.g. Porter, 1969; Hill, Noakes and Lowe, 1970; Leat, 1970). The reticulo-rumen and omasum appear to be relatively non-functional and are underdeveloped compared to the abomasum at birth (Wardrop and Coombe, 1961). The forestomachs show developmental changes during the first three weeks of life but no further development in size or digestive function occurs until substantial quantities of solid food are eaten and a microbial population is established (Wardrop, 1960). On the other hand, the abomasum changes little after birth, apart from the rapid maturation of gastric glands during the first day of life (Hill et al., 1970). Histologically, however, the most extensive changes in the fore-stomachs occur during the last 50 days of fetal life (Wardrop, 1961). A catheterisation technique for obtaining fluid samples from the reticulo-rumen and abomasum of the fetal sheep during the last third of gestation was devised in order to study the fetal digestive tract.

Although the forestomachs and abomasum do not appear to be important functionally until after birth, a role in fluid dynamics in the fetus cannot be discounted. The sheep fetus swallows 250-480 ml of fluid per day (Bradley and Mistretta, 1973) but produces little meconium. Its gastro-intestinal tract must therefore absorb both water and solutes. In the anaesthetised animal the abomasum and intestines absorb fluid (Wright and Nixon, 1961). Whether the rumen modifies the fluid entering it from the amniotic sac, however, is unknown. This possibility has been examined by comparing the osmolalities and the concentrations of Na and K of the amniotic, ruminal and abomasal fluids obtained between 90 and 140 days of gestation by means of a catheterisation technique. The pH was also measured to assess whether acid secretion by the abomasum starts before birth.

6.2 EXPERIMENTAL METHODS

6.2.1 Animals

Five sheep were used, three carrying single and two carrying twin fetuses of known conceptual age. The rumen and abomasum of one fetus in each ewe were catheterised as described in Chapter Two.

6.2.2 Measurements

Na and K concentrations were determined directly in maternal plasma and amniotic fluid and in diluted samples of ruminal and abomasal fluids. The viscosity of the ruminal and abomasal fluid prevented volumetric analysis, so a known weight of sample was diluted with distilled water and electrolyte concentrations were expressed on a weight basis. There were no differences in the

concentrations of Na or K determined on a volume or weight basis in 10 maternal plasma and 10 amniotic fluid samples. All concentrations are reported in mmol/kg fluid in this chapter. Sample volumes did not permit the concentrations of other substances to be measured. For simplicity the fluid from the reticulo-rumen is referred to as ruminal fluid.

6.3 RESULTS AND DISCUSSION

Five fetuses were catheterised at 87-96 days gestational age and were born between 145-147 days which is within the range observed in uncatheterised sheep. The course of parturition, birth weights of the lambs (3.1-4.9 kg) and their growth rates to eight weeks of age (1.0-2.7 kg/week) were normal. The ruminal and abomasal fluids were viscous and in three fetuses the 0.86mm (i.d.) catheters blocked after 25-30 days while in two fetuses the 1.00 mm (i.d.) catheters remained patent for 40-50 days.

Fluid from the rumen appeared colourless while that from the abomasum was often a yellow-orange colour.

Osmolalities and Na concentrations were generally higher and K concentrations lower in amniotic fluid (Table 6:1). The difference in K was less marked before 120 days of gestation than subsequently. As fetal plasma samples were not taken, values for the osmolality and the concentrations of Na and K of fetal plasma were derived from the maternal values (Table 6:1) using the maternal/fetal ratio reported by Mellor and Slater (1971). A comparison using these derived values suggests that amniotic fluid was hypotonic to fetal

TABLE 6:1

Mean ($\pm$SD) values for osmolality (mOsm/kg water) and sodium and potassium concentrations (mmol/kg fluid) in maternal plasma and in amniotic fluid (AMF), ruminal fluid (RU) and abomasal fluid (ABO) of five fetuses between 91 and 140 days gestation, except for RU which was obtained between 91 and 130 days. The significance of differences between AMF and RU, RU and ABO and maternal plasma and ABO are shown.

	Gestational Age Range (days)					
	91-100	101-110	111-120	121-130	131-140	91-140
Osmolality						
AMF	270$\pm$23 (30)	264$\pm$29 (39)	284$\pm$24 (33)	258$\pm$22 (21)	247$\pm$18 (11)	264$\pm$22 (134)
Ru	320$\pm$36 (25)***	320$\pm$54 (40)***	329$\pm$63 (30)***	347$\pm$36 (8)***	-	326$\pm$52 (103)***
Abo	332$\pm$52 (17)^{ns}	331$\pm$47 (28)^{ns}	348$\pm$69 (27)^{ns}	344$\pm$48 (9)^{ns}	320$\pm$40 (12)***	337$\pm$55 (93)^{ns}
MP						279$\pm$10 (142)***
Sodium						
AMF	122$\pm$9 (27)	116$\pm$6.4 (24)	123$\pm$9.4 (19)	118$\pm$6.4 (25)	110$\pm$9.9 (10)	118$\pm$1.8 (105
Ru	131$\pm$5.9 (20)***	128$\pm$20 (25)**	137$\pm$27 (16)***	145$\pm$25 (11)***	-	136$\pm$19.4 (72)***
Abo	136$\pm$6.9 (16)*	134$\pm$18.4 (24)^{ns}	143$\pm$14 (22)^{ns}	145$\pm$18.2 (7)^{ns}	132$\pm$17.2 (7)***	140$\pm$17.8 (69)^{ns}
MP						145$\pm$1.5 (123)**
Potassium						
AMF	8$\pm$2.8 (27)	7.4$\pm$1.5 (24)	7.34$\pm$1.7 (19)	8.6$\pm$1.5 (25)	9.0$\pm$1.6 (10)	8.06$\pm$1.6 (105)
Ru	7.47$\pm$2.8 (20)^{ns}	5.8$\pm$4.4 (25)^{ns}	5.8$\pm$0.7 (16)^{ns}	6.1$\pm$1.0 (19)***	-	6.29$\pm$1.75 (72)*
Abo	7.1$\pm$2.7 (16)^{ns}	5.7$\pm$1.2 (24)^{ns}	5.68$\pm$0.8 (22)^{ns}	6.1$\pm$1.2 (7)^{ns}	6.2$\pm$1.4 (7)***	6.0$\pm$1.6 (69)^{ns}
MP						5.15$\pm$0.12 (123)^{ns}

Maternal plasma (MP) osmolality was significantly higher ($P < 0.05$) than that of AMF. ^{ns} not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The number of observations are given in brackets.

plasma which was itself hypotonic to both ruminal and abomasal fluid, and that fetal plasma, ruminal fluid and abomasal fluid concentrations of Na and K would not have been significantly different.

These data demonstrate that the composition of amniotic fluid was modified during its passage between the amniotic sac and the rumen. Fetal lung fluid may have been involved, because 100-200 ml are secreted each day (Adamson, Brodecky, Lambert, Maloney, Richie and Walker, 1975), and even if 'bouts' of swallowing (Bradley and Mistretta, 1973) do not coincide with opening of the laryngeal sphincter and the associated flow of lung fluid into the pharynx (Adams, Desilets and Towers, 1967), lung fluid that subsequently accumulated in the pharyngeal and buccal cavities would mix with amniotic fluid during the next period of swallowing. Such a process could account for the different electrolyte compositions of ruminal and amniotic fluid, but cannot explain the hypertonicity of ruminal fluid, because lung fluid and plasma have similar Na and K concentrations and are isotonic (Adamson, Boyd, Platt and Strang, 1969). Therefore, unless the fetal sheep secretes saliva that is markedly hypertonic to plasma (and there is apparently no information on this point although adult saliva is isotonic (Kay, 1960)), the high osmolality of ruminal fluid must result from net secretion of solutes in the ruminal lumen or net absorption of water from it, or both. Further evidence for the presence of such ruminal processes is the greater day to day fluctuations in the osmolality of ruminal fluid compared to amniotic fluid (indicated in Table 6:1 by the different SDs), which suggests different lengths of exposure of the fluid to secretory or absorptive processes in the rumen. Since swallowing occurs intermittently (Bradley and Mistretta, 1973) the interval between

sampling and the last period of swallowing in the present fetuses would have varied. The compositions of the ruminal and abomasal fluids were not significantly different (Table 6:1), so any further alterations to fluid composition by the omasum and abomasum were apparently less marked.

From 87 to 143 days gestational age the pH of the amniotic, ruminal and abomasal fluids remained relatively constant with mean ($\pm$SD) values of 6.96$\pm$0.17 (144), 6.95$\pm$0.15 (77) and 6.98$\pm$0.15 (49), respectively. Therefore, hydrogen ion secretion by the fetal abomasum did not exceed the buffering capacity of abomasal fluid during this period.

Although the viscous nature of the fluid obtained made sampling and analytical measurements difficult, the catheterisation technique itself was successful. The catheters provide an ideal means of introducing substances, such as radio-active materials, into the fetal forestomachs or abomasum in order to study fetal gut function in the conscious unstressed animal.

6.4 CONCLUSIONS AND COMMENTS

1. The composition of amniotic fluid is modified during its passage between the amniotic sac and the rumen.
2. Further alteration to swallowed fluid by the omasum and abomasum is apparently less marked.
3. Acid secretion by the abomasum before birth did not exceed the buffering capacity of abomasal fluid.
4. Catheters provide an ideal means of introducing substances into

the fetal forestomachs or abomasum but sampling is difficult due to the viscous nature of the fluid obtained.

5. Further work could involve the use of larger diameter catheters, analysis of other constituents and a more detailed investigation of changes in fluid composition during its passage from the amniotic sac to the rumen and changesin the rumen.

CHAPTER SEVEN

THE IONIC COMPOSITION OF ALLANTOIC FLUID AND FETAL URINE DURING INFUSION OF ACTH OR CORTICOSTERONE INTO FETAL SHEEP.

7.1 INTRODUCTION

In addition to permitting fluid withdrawal, the technique of 'chronic' catheterisation allows substances to be injected or infused into the fetus. Fetuses were infused through indwelling catheters during the course of the studies described in this chapter. The work was carried out conjointly with Dr. D.J.Mellor and consisted of pilot experiments associated with research that was in progress when the author joined the laboratory.

It has been suggested that in the sheep, fetal corticosteroids by their mineralocorticoid effect act on ion pumps in the chorio-allantois and fetal kidneys, altering the Na and K concentrations of allantoic fluid and fetal urine. This hypothesis was based on the following observations:

1) In normoglycaemic ewes during the 5-10 days before birth at term there is a marked decrease in Na and increase in K concentrations of allantoic fluid (Mellor and Slater, 1971) and during the same period the Na/K concentration ratio (Na/K ratio) of fetal urine decreases more rapidly (Mellor and Slater, 1972a).

2) Electrolyte concentrations in allantoic fluid and fetal urine in individual animals change in parallel (Mellor and Slater, 1972a, 1974), and are consistent with a simultaneous action of mineralocorticoids on the chorioallantois and fetal kidneys.

3) Apart from aldosterone, cortisol and corticosterone are the main

corticosteroids secreted by the adrenal gland of the fetus (e.g. Jones, Jarrett, Vinson and Potter, 1964; Alexander, Britton, James, Nixon, Parker, Wintour and Wright, 1968a; Wintour, Brown, Denton, Hardy, McDougall, Oddie and Whipp, 1975).

4) Concentrations of corticosteroids in fetal plasma increase markedly during the 5-10 days before birth (e.g. Bassett and Thorburn, 1969; Comline, Nathanielsz, Paisey and Silver, 1970; Drost, Kumagai and Guzman, 1973; Liggins, Fairclough, Grieves, Kendall and Knox, 1973) at the time when the changes in electrolyte concentrations occur.

Despite the above evidence, a direct relationship between changes in fetal adrenocortical secretions and the composition of allantoic fluid and fetal urine has yet to be established. Infusions of adrenocorticotrophic hormone (ACTH) or corticosterone into individual fetuses in normoglycaemic ewes were carried out to test this association. In hypoglycaemic ewes maximum K and minimum Na concentrations in allantoic fluid can be approached as early as 110 days in gestation, and can be maintained until term or until normoglycaemia is restored (Mellor and Slater, 1972a). In order to eliminate any effect of changes in plasma glucose concentrations, only the results from ewes showing relatively constant plasma glucose concentrations before and during infusion have been used in the present chapter. Changes in fetal urine composition in one goat before abortion are reported.

7.2 EXPERIMENTAL METHODS

7.2.1 Animals

Two single-bearing Welsh Mountain ewes, four Scottish Blackface

ewes (one twin-bearing) and one goat with a single fetus were used.

7.2.2 Catheters

Catheters were inserted into the allantoic sac and an umbilical vein of one fetus in each of five ewes at 103-121 days in gestation, into the bladder and peritoneal cavity of one fetus in one ewe at 85 days and into the bladder of the goat fetus at 97 days.

7.2.3 Infusions

Details of the method of infusion are outlined in Chapter Two.

Synthetic ACTH (Synacthen, Ciba Laboratories, Sussex) was dissolved in sterile 5% $^{w}/_{v}$ glucose solution (BDH) which was infused into the umbilical vein (three fetuses) or fetal peritoneal cavity (one fetus) at 0.67 ml/hr with ACTH concentrations (1.5 mg/l or 6.0 mg/l) allowing delivery rates of 1.0 or 4.0 µg/hr.

Synthetic corticosterone (Koch-Light Laboratories Ltd.) dissolved in 1ml ethanol was added to sterile 5% $^{w}/_{v}$ glucose solution to give a concentration of 8.57 mg/l which was infused into the umbilical vein of two fetuses at 0.7 ml/hr to allow a delivery rate of 6 µg/hr. This was approximately one third of an estimated rate of corticosterone secretion (20 µg/hr) by the adrenal gland, which was calculated from estimates of secretion rates obtained from eight exteriorised fetuses of 110-134 days gestational age (Alexander et al., 1968a).

Control infusions of 5% $^{w}/_{v}$ glucose solution at 0.7 ml/hr were not conducted here as Mellor (unpublished data) examined the effects in five sheep and found that the electrolyte compositions of allantoic fluid and fetal urine were not affected.

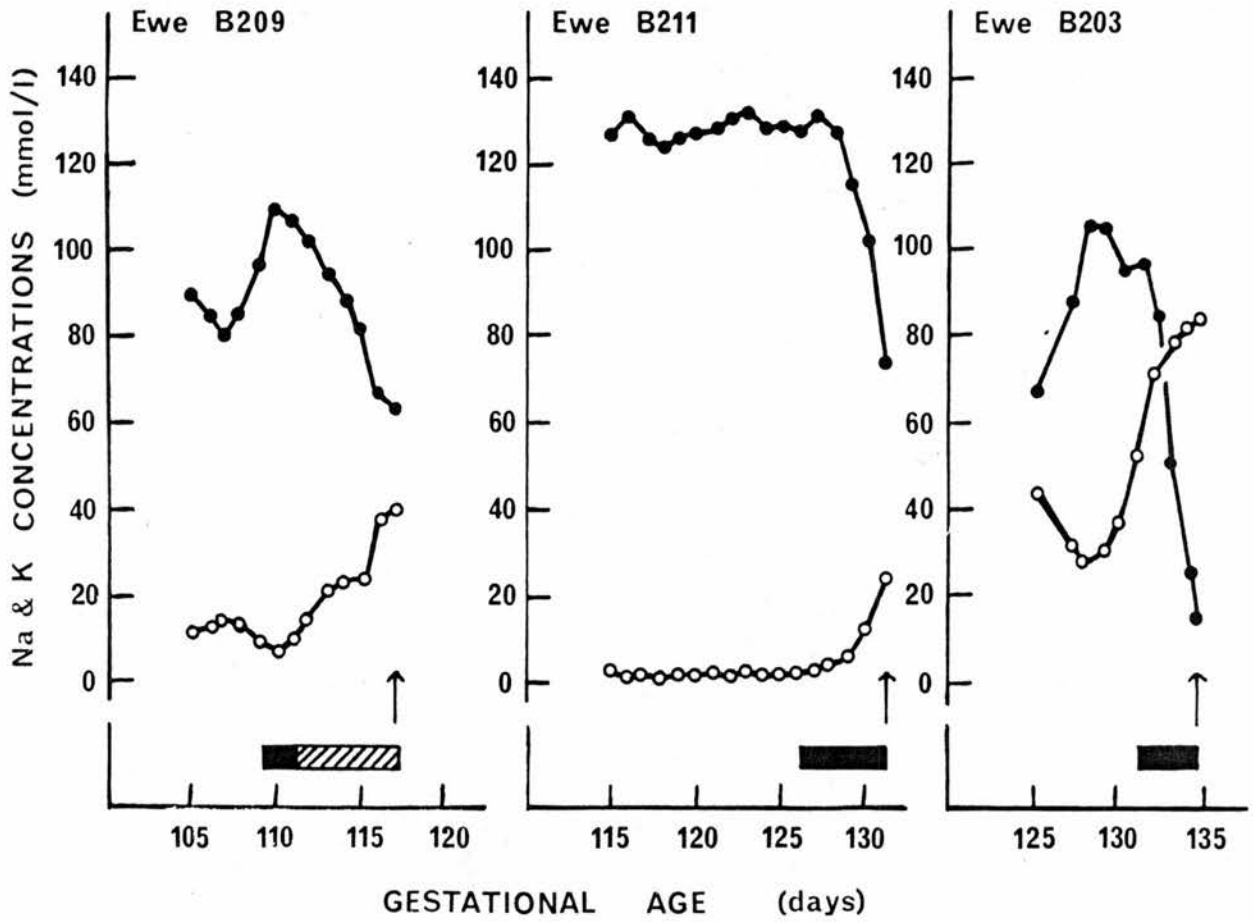

Figure 7:1

Changes in sodium (●) and potassium (○) concentrations in allantoic fluid from three fetuses which were infused intravenously with ACTH at rates of 1.0 µg/hr (solid bar) or 4.0 µg/hr (striped bar) until delivery (arrow). Gestational ages at operation were 103 days (B209), 107 days (B211) and 121 days (B203).

7.3 RESULTS

7.3.1 ACTH infusions

ACTH infusions were allowed to continue until premature delivery. The lambs were all alive at birth, which occurred at 110 (B216), 117 (B209), 131 (B211) and 135 (B203) days of gestation. The lambs from ewes B216 and B209 and the non-infused twin from ewe B203 died within 1 hr, but the other two survived for at least 35 days. The birth weights of 1.3 kg (B216), 1.7 kg (B209), 2.6 kg (B211), 2.3 kg (non-infused: B203) and 2.6 kg (infused: B203) were normal for the stage of gestation and the weights of the adrenal tissue of the dead lambs (320-715 mg) were within the established ranges for infused and non-infused fetuses delivered prematurely in this way (Liggins, 1968; Nathanielsz, Comline, Silver and Paisey, 1972).

In three fetuses (from B209, B211 and B203), ACTH infusion into an umbilical vein was associated with a fall in Na and a rise in K concentrations of allantoic fluid which continued until birth (Fig.7:1). Immediately before and during infusion in each animal the glucose concentrations of maternal plasma remained relatively constant (B209, 460-520 mg/l; B211, 500-550 mg/l; B203, 370-410 mg/l). Similar electrolyte changes were observed in allantoic fluid from three other ewes which delivered live lambs at 122, 125 and 127 days in gestation after ACTH infusions (1.0 μg/hr) into an umbilical vein lasting three days. The results from these ewes were not reported as plasma glucose concentrations decreased during infusion.

In one fetus (from ewe B216) ACTH infusion into the fetal

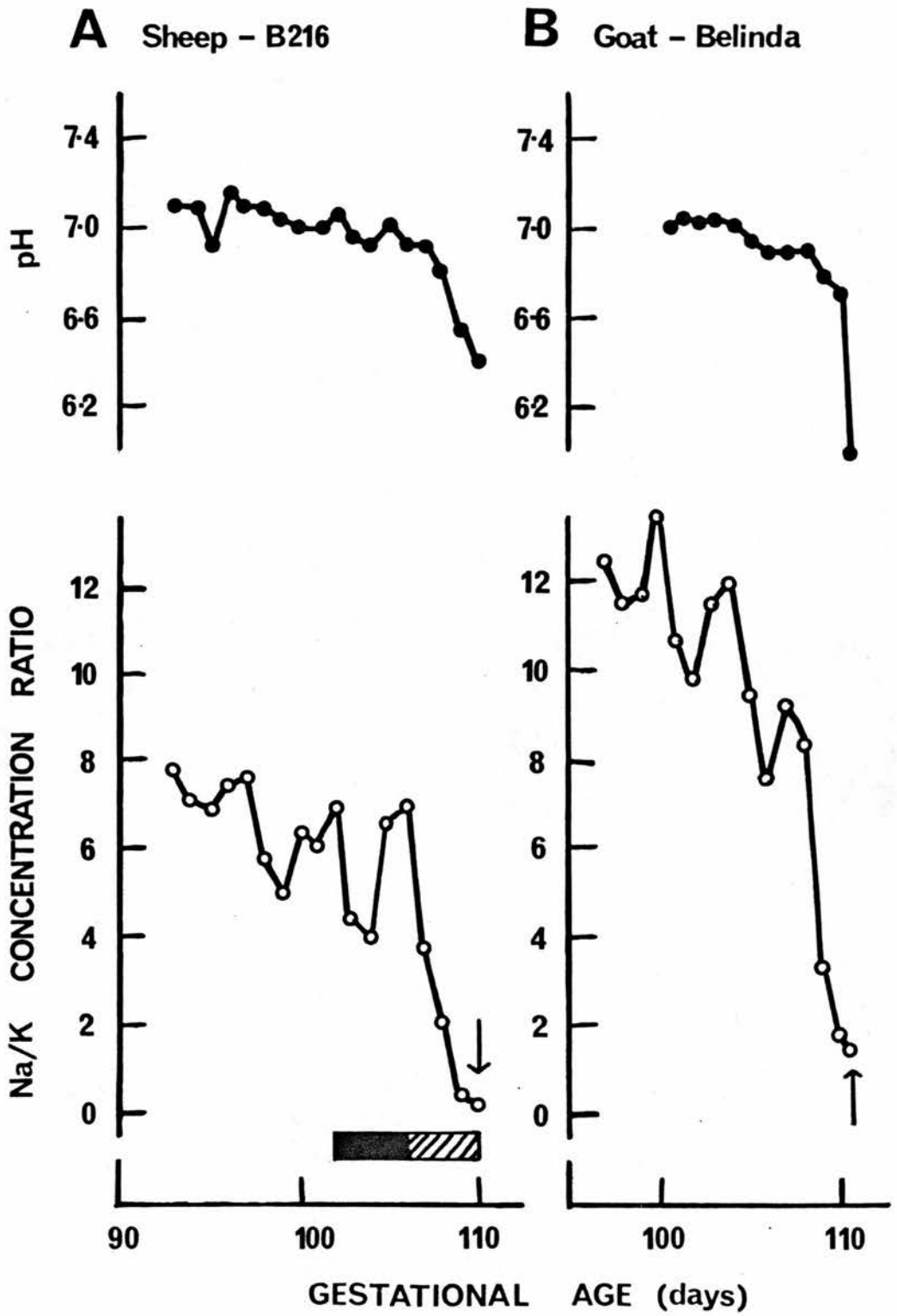

Figure 7:2

Changes in pH and Na/K concentration ratio in fetal urine from: A. One sheep fetus which was infused intraperitoneally with ACTH at a rate of 1.0 µg/hr (solid bar) or 4.0 µg/hr (striped bar) until delivery (arrow) and B. One goat fetus before abortion (arrow). Gestational ages at operation were 85 days (B216) and 97 days (Belinda).

peritoneal cavity was associated with a fall in Na and a rise in K concentrations of fetal urine, as reflected by a decrease in the Na/K ratio. At the same time there was a marked fall in urine pH (Fig.7:2A). These changes were similar to those observed in the fetal urine of sheep before natural delivery (Mellor and Slater, 1972a b). Maternal plasma glucose concentrations remained between 450-500 mg/l in this ewe during infusion. Osmolality and the concentrations of Na and K in fetal urine before birth are likely to be affected by the increase in fetal plasma arginine vasopressin (AVP) concentration which occurs during the last four days of pregnancy (Alexander, Bashore, Britton and Forsling, 1974b). As a result, changes in Na and K concentrations in fetal urine were expressed as Na/K ratio in all cases.

7.3.2 Corticosterone infusions

Infusion of corticosterone was started at 125 days (WM 599) or 128 days (WM 597) in gestation and was allowed to continue for five days in each ewe. Birth occurred at 145 days (WM 599) and 151 days (WM 597) in gestation. The two infused fetuses were alive at birth and had usual birth weights (2.6 kg and 2.5 kg) and growth rates to weaning for Welsh Mountain lambs.

In one ewe (WM 597) the corticosterone infusion was associated with an acceleration of a fall in Na and a rise in K concentrations in allantoic fluid. These trends were reversed when the infusion stopped (Fig.7:3). No significant changes in allantoic fluid composition occurred in the other ewe (WM599) during or after corticosterone infusion (Fig.7:3)

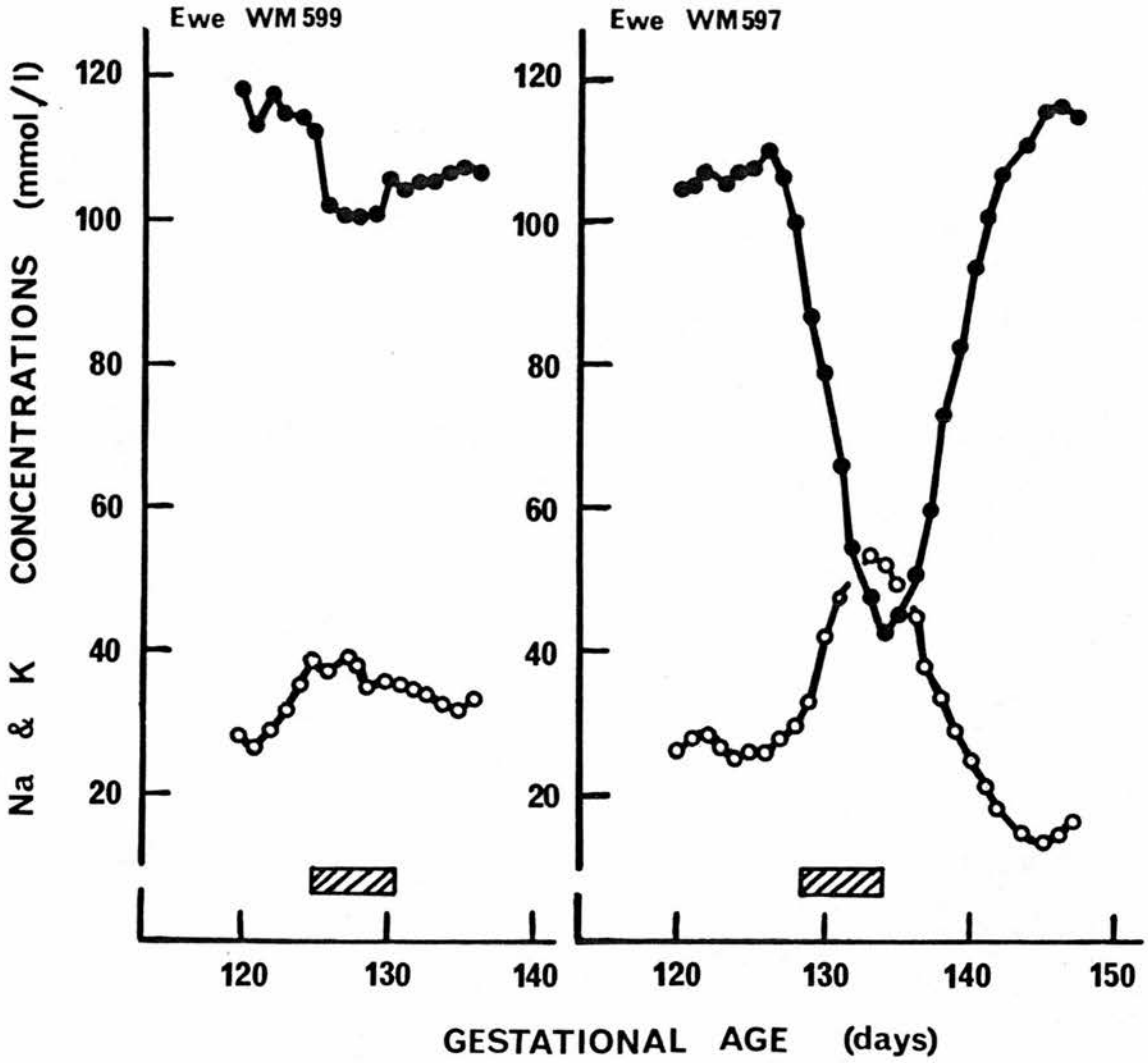

Figure 7:3

Changes in sodium (●) and potassium (○) concentrations of allantoic fluid from two fetuses which were infused intravenously with corticosterone at a rate of 6 µg/hr (striped bar). Birth occurred at 145 days (WM599) and 151 days (WM597) in gestation. Gestational ages at operation were 112 days (WM599) and 118 days (WM597).

7.3.3 The composition of goat fetal urine before abortion

Abortion occurred 14 days after surgery at 110 days of gestation in one goat (Belinda). Its fetus was alive at birth (weight 0.85 kg) but died within a few minutes. A decrease in pH and the Na/K ratio of fetal urine occurred during the last few days before abortion (Fig.7:2B).

7.4 DISCUSSION

As maternal plasma glucose concentrations remained relatively constant during ACTH infusion, the infusion is the likely cause of the decrease in the Na/K ratio in allantoic fluid and fetal urine. Infusion of ACTH into fetal sheep at 4-10 μg/hr causes adrenal gland hypertrophy, a progressive increase in the secretion and plasma concentrations of corticosteroids and premature parturition after 4-7 days (Liggins, 1968; Liggins, Grieves, Kendall and Knox, 1972; Bassett and Thorburn, 1973; Liggins et al., 1973). Similar changes occur in fetuses during the 4-10 days before natural parturition (e.g. Bassett and Thorburn, 1969, 1973; Comline et al., 1970; Drost et al., 1973; Mellor et al., 1975b). Although corticosteroid concentrations of fetal plasma were not measured in this study, birth occurred prematurely, so it is likely that plasma concentrations increased as a result of ACTH stimulation of fetal corticosteroid secretion. Since ACTH is not known to act directly on ion pumps, the increased corticosteroid concentrations were probably responsible for the decrease in Na/K ratio in allantoic fluid and fetal urine. Further evidence for an association between fetal corticosteroids and ionic changes in the fluids has since been obtained. An increase in fetal plasma corticosteroid concentrations (which started at 134-136 days

of gestation) and a decrease in the Na/K ratio of allantoic fluid occurred simultaneously in five ewes before birth (Mellor et al., 1975b).

An association between corticosteroid concentrations and the Na/K ratios suggests a mineralocorticoid action of the adrenal steroids. Cortisol and corticosterone are the major glucocorticoids secreted by the fetal adrenal gland near term (Jones et al., 1964; Alexander et al., 1968a; Alexander, Britton, Nixon, Ratcliffe and Redstone, 1973b; Wilson, Thomas, Pierrepoint and Griffith, 1973; Jones, 1975; Jones, Boddy, Robinson and Ratcliffe, 1975), but corticosterone is thought to have mineralocorticoid activity as well (e.g. Liggins, 1969a). However, the results of the corticosterone infusion were rather inconclusive. Either its mineralocorticoid effect is minimal or the infusion did not equal its secretion rate by the fetal adrenal. Clearly, higher infusion rates in more animals need to be carried out before its action can be established.

Aldosterone is secreted by the ovine fetal adrenal gland (Alexander et al., 1968a; Wintour et al., 1975), but it is not known whether aldosterone release is mediated through ACTH secretion in sheep. Wintour et al. (1975) found that after 120 days gestational age increased production of aldosterone from the fetal adrenal gland occurs in vitro in response to an increase in K concentration in the incubation medium. In addition, the renin-angiotensin system of the fetus appears to be fairly well developed and functions independently of the mother during the last third of gestation (Mott, 1973, 1975). In adult sheep corticosterone and cortisol infusions have little

effect on the Na/K ratio of parotid saliva or urine in adrenalectomised or functionally adrenalectomised sheep, respectively, whereas an aldosterone infusion reverses the trend in the Na/K ratio (Blair-West, Coghlan, Denton, Goding and Wright, 1963; Foster and Harrison, 1975). So, in the ovine fetus, the changes in Na/K ratio in allantoic fluid and fetal urine before birth appear more likely to be due to an effect of aldosterone than to corticosterone. However, a combined action of these corticosteroids or others (e.g. 11-deoxycortisol: Thomas, Wilson, Pierrepoint, Cameron and Griffith, 1976; 11-deoxycorticosterone: Wintour et al., 1975) which are secreted during the increase in fetal adrenal activity before birth, is also possible. But, until the glucocorticoid and mineralocorticoid effects of cortisol, corticosterone and aldosterone are examined in the chronic preparation the relative importance of the effects of each steroid on allantoic fluid and fetal urine will remain uncertain.

The striking similarity between the ionic and pH changes in fetal urine from the goat that aborted 13 days after surgery and the sheep that gave birth prematurely because of the ACTH infusion (Fig.7:2) suggests that abortion in this goat was associated with an increase in fetal plasma corticosteroid concentrations. Such a corticosteroid increase has been observed in a fetal goat which aborted 13 days after operation (Thorburn et al., 1972).

Maternal hypoglycaemia at any stage between about 90 days of pregnancy and the prepartum increase in fetal adrenocortical activity induces changes in the ionic composition of allantoic fluid of the same or a greater magnitude than those observed here, without causing

premature delivery (Mellor and Slater, 1971, 1972a). It seems, therefore, that the marked rise in fetal plasma corticosteroids observed before natural and ACTH induced parturition is not necessary to stimulate these ionic changes in allantoic fluid. In fact, fetal plasma corticosteroid concentrations after about 90 days of gestation remain low until the marked prepartum rise (Bassett and Thorburn, 1969, 1973; Drost et al., 1973; Liggins et al., 1973; Wintour et al., 1975). Therefore, the ion pumping mechanisms in the chorioallantois (Mellor, 1970b) and the fetal kidneys probably respond to small variations in plasma concentrations of corticosteroids. Mellor and Slater (1971, 1972a) suggested that the ionic changes observed were due to maternally induced fetal hypoglycaemia causing increases in fetal corticosteroid concentrations. An inverse relationship between glucose and corticosteroid concentrations of fetal plasma has been observed under various experimental conditions (Liggins et al., 1973; Jones, 1976; Mellor, Matheson and Small, 1977) and agrees with this suggestion. However, other workers have been unable to find an association between maternal or fetal hypoglycaemia and changes in fetal corticosteroids (Bassett and Madill, 1974a; Shelley, Bassett and Milner, 1975). Therefore, the possibility that maternal corticosteroids are important must not be overlooked. During hypoglycaemia maternal plasma corticosteroid concentrations in sheep show a marked increase (Fig.4:1; Bassett and Madill, 1974a) and maternal corticosteroids can apparently enter the fetal circulation (Beitins et al., 1970; Dixon et al., 1970; Liggins et al., 1973). If maternal corticosteroids cross the placenta in amounts which are sufficient to act on the chorioallantois during maternal hypoglycaemia, they could be directly responsible for changes in electrolyte

concentrations in allantoic fluid before a prepartum rise in fetal plasma corticosteroids occurs. Alternatively, maternal corticosteroids may act directly on the endometrial surface of the chorion.

Prenatal activation of the fetal adrenal glands appears to be an important factor in determining survival of the neonate. Viability was greater in lambs delivered prematurely as a result of ACTH infusion than in lambs of a similar maturity delivered by Caesarian section (Alexander, Thorburn, Nicol and Bell, 1972a). Liggins (1969b) suggested, and it has since been confirmed (De Lemos, Shermeta, Knelson, Kotas and Avery, 1969, 1970; Platzker, Kitterman, Clements and Tooley, 1972) that increasing levels of corticosteroids induce surfactant production in the fetal lungs. Corticosteroids have also been implicated in changes in the sensitivity of pancreatic β-cells to glucose at birth (Bassett, Madill, Nicol and Thorburn, 1973; Shelley et al., 1975) and to the replacement of brown with white adipose tissue which is initiated at birth (Alexander, Nicol and Thorburn, 1973c). The survival of the infused twin but not its non-infused littermate following ACTH induced premature parturition in the sheep (B203) in the present work, and in twin-bearing goats (Thorburn et al., 1972), is in accord with the above findings.

7.5 CONCLUSIONS AND COMMENTS

1. A marked increase in fetal plasma corticosteroid concentrations is the likely cause of the changes in Na and K concentrations of allantoic fluid observed before ACTH induced premature delivery, in this study, and before natural birth at term (others' work).
2. The increase in fetal plasma corticosteroid concentrations is

the likely cause of the simultaneous decrease in the pH and Na/K ratio in fetal urine before natural and ACTH induced birth.

3. Changes in the composition of fetal urine before abortion in one goat were consistent with a rise in the corticosteroid concentrations in plasma assuming a similar effect of fetal corticosteroids on the composition of fetal urine in the goat as is found in sheep.

4. Prenatal activation of the fetal adrenal gland is important to the survival of the neonate.

5. It was not possible to say whether the fluctuations in Na and K concentrations of allantoic fluid before the marked prepartum changes were due to smaller changes in corticosteroid levels than those observed before delivery. In order to answer this question fetal plasma corticosteroid and allantoic fluid and fetal urine electrolyte concentrations would have to be examined simultaneously in individual fetuses before the prepartum increase in fetal plasma corticosteroids occurs.

6. Infusions of corticosterone and other fetal adrenal steroids at differing rates into the fetal circulation would be necessary if the relative importance of the effects of each of the adrenal steroids on the composition of allantoic fluid and fetal urine is to be established.

CHAPTER EIGHT

BEHAVIOUR AND CHANGES IN PLASMA COMPOSITION IN NEONATAL SHEEP AND GOATS DURING THE FIRST 16 HOURS AFTER BIRTH

8.1 INTRODUCTION

The work described in this chapter is concerned with the neonatal lamb and kid. In order to set the scene for the studies a brief account of the current ideas relating to parturition in sheep and goats is relevant since various events which occur during parturition are essential for the survival of the newborn. There are several detailed accounts of the changes occurring in the mother and fetus at the end of gestation which ultimately lead to expulsion of the fetus (e.g. Thorburn et al., 1972; Ash, Challis, Harrison, Heap, Illingworth, Perry and Poyser, 1973; Liggins et al., 1973; Currie, 1974; Challis and Thorburn, 1975).

As a result of an as yet unknown stimulus, but one apparently dependent upon maturation of the fetal hypothalamo-pituitary-adrenal axis (Challis and Thorburn, 1975), an increase in the secretion of corticosteroids from the fetal adrenal glands occurs (Nathanielsz et al., 1972; Liggins et al., 1973). This results in a rise in fetal plasma corticosteroid concentrations most rapidly in the 2-3 days immediately before birth reaching highest values near or at delivery (e.g. Bassett and Thorburn, 1969; Comline et al., 1970; Drost et al., 1973; Liggins et al., 1973; Currie, 1974; Mellor et al., 1975b). The rise in fetal plasma corticosteroids may be partly due to an increase in fetal transcortin-binding capacity (Fairclough and Liggins, 1975). In addition, an abrupt increase in

maternal plasma oestrogen concentrations occurs at parturition (Challis, 1971; Challis and Linzell, 1971; Thorburn et al., 1972; Currie, Wong, Cox and Thorburn, 1973; Currie, 1974). The oestrogens appear to originate in the feto-placental unit (Ainsworth and Ryan, 1966), since at the same time a large increase in fetal plasma oestrogen sulpho-conjugates also occurs (Currie et al., 1973). Although the processes involved in oestrogen formation have yet to be clarified (Challis and Thorburn, 1975) the prepartum increase in fetal plasma concentrations of corticosteroids has been implicated (Ash et al., 1973; Currie et al., 1973). In sheep, the decrease in maternal progesterone concentrations usually observed before parturition has also been attributed to an effect of fetal corticosteroids on placental steroidogenesis (Liggins, 1969ab; Anderson, Flint and Turnbull, 1975). However, in goats, fetal corticosteroids may act directly, or indirectly via an effect on oestrogens, to induce the release of placental PGF into the utero-ovarian veins, thus causing luteolysis and a rapid decrease in maternal plasma progesterone concentrations (Thorburn et al., 1972; Currie, 1974; Currie and Thorburn, 1973). The changes in the concentrations of oestrogens and progesterone in maternal plasma may provide a direct stimulatory influence on the myometrium (Currie, 1974; Jones and Knifton, 1977). The concentration of PGF, which is a major determinant of uterine contractility (Liggins et al., 1973; Challis 1974; Robinson and Thorburn, 1974; Goldberg and Ramwell, 1975), increases in uterine blood during the first stage of labour (Currie, 1974) apparently as a result of increased production of PGF and its release into the maternal plasma and myometrium (Challis and Thorburn, 1975). PGF synthesis in the placenta may be regulated by

the ratio of progesterone to oestrogen within the feto-placental unit, and synthesis in the myometrium may be a result of stretching of the tissue during contraction (Challis and Thorburn, 1975). A marked increase in maternal plasma concentrations of oxytocin occurs during stage two of labour (Chard, Boyd, Forsling, McNeilly and Landon, 1970; McNeilly, Forsling and Chard, 1971). The stimulus for oxytocin release in the ruminant may act through neural pathways (Chard, 1973ab) although the substantial amount of PGF in the systemic circulation during labour (Gillespie, Brummer and Chard, 1972) may, at least in man, also stimulate oxytocin release (Chard, 1973ab; Currie, 1974; Goldberg and Ramwell, 1975). Oxytocin enhances uterine contractions which expel the fetus in the final stages of parturition (Chard, 1972). PGF concentrations in the uterine veins also increase markedly during stage two labour and Flint, Forsling, Mitchell and Turnbull (1975) and Roberts and McCracken (1976) suggest that an action of oxytocin may be to increase myometrial PGF synthesis. This may in turn enhance myometrial sensitivity to the effects of oxytocin (Liggins et al., 1973). However, increased synthesis of PGF may not be an essential intermediate step in the activation of the myometrium by oxytocin (Roberts and McCracken, 1976).

Physical signs of labour are not usually apparent until about 12 hrs before delivery. Labour gradually intensifies during the first stage by increases in the frequency and strength of uterine contractions. In stage two delivery of the fetus is completed with the assistance of maternal abdominal straining which is closely synchronised with the uterine contractions (Hindson et al., 1965; Currie, 1974).

Changes in maternal plasma hormones may also be concerned with establishing lactation. In goats, the marked prepartum change in oestrogens appears to be associated with increased secretion of immunoglobulins, and increased plasma cortisol and prolactin concentrations may provide a lactogenic trigger (Fleet, Goode, Hamon, Laurie, Linzell and Peaker, 1975). In sheep, oestrogen as well as prolactin may be important in stimulating lactogenesis (Hartman, Trevethan and Shelton, 1973; Fulkerson, Hooley, McDowell and Fell, 1976) and it has been suggested that the increase in mammary blood flow several hours before delivery is related to the increase in fetal adrenocortical activity (Burd, Lemons, Makowski, Battaglia and Meschia, 1975) and that the decrease in progesterone concentration in maternal plasma after birth is a trigger for the change from colostrum to true milk secretion by the mammary gland (Currie, 1974).

Much has been written in favour of the increased secretion of fetal corticosteroids, in sheep and goats, being necessary for the initiation of parturition. Although impoartant for survival of the neonate (see Chapter Seven), their role in parturition needs careful consideration. Evidence for a direct action of ovine fetal corticosteroids came in the 1960's and was based on prolongation of pregnancy in ewes carrying fetuses with abnormalities of the hypothalamo-pituitary-adrenal axis (Binns, James, Shupe and Everett, 1963; Holm, 1967; Basson, Morgenthal, Bilborough, Marais Kruger and Van der Merwe, 1969; Kennedy, 1971) or with experimentally produced ablations of the hypothalamus, pituitary or adrenal glands (Liggins, Kennedy and Holm, 1967; Drost and Holm, 1968; Liggins et al.,

1973). Associated with these studies was the finding that premature parturition was caused by the administration of ACTH, cortisol or a synthetic glucocorticoid (dexamethasone) to the fetus (Liggins, 1969a; Liggins et al., 1973). These experiments were followed by the demonstration in sheep of a prepartum increase in the concentrations of fetal plasma corticosteroids (e.g. Bassett and Thorburn, 1969) which preceded all other hormone changes (Currie, 1974). Similar findings were then reported in goats (Thorburn et al., 1972), and van Rensburg (1971) found that adrenal hyperplasia was associated with premature parturition in habitually aborting goats. When considered in toto this evidence seems convincing, however, the physiological significance attributed to some of these observations may be questioned.

Ablation studies have some defects which hinder unequivocal interpretation of the results: ultrastructural changes in the placenta of the ewe occur after fetal hypophysectomy or adrenalectomy (Barnes, Comline, Silver and Steven, 1976a). Damage to the hypothalamus and pituitary gland will inevitably affect hormone systems other than the pituitary-adrenal axis, and ablation of the adrenal cortex disturbs metabolism (Barnes, Comline and Silver, 1976b, 1977) and probably developing enzyme systems (Alexander et al., 1973b) and, being associated with a high incidence of fetal death (Liggins, Holm and Kennedy, 1966; Drost and Holm, 1968; Barnes et al., 1977), may have several unknown but vital roles which indirectly affect the birth process.

Hormone infusions have in some cases been at excessive rates and

their physiological actions can be questioned. Although the minimum rate of infusion of synthetic ACTH required to cause premature parturition in sheep has not yet been established, the lowest reported rate used (1 μg/hr: Chapter Seven) when infused into adult sheep maintained plasma corticosteroid concentrations of 80-140 ng/ml for 3-5 days (Mellor, unpublished data). In fetal goats, ACTH infusion (10 μg/hr) for more than five days was sometimes necessary to cause premature parturition and had apparently deleterious effects on the viability of the fetus (Currie, 1974). Synthetic ACTH itself may have a pharmacological action since the fetal adrenal cortex appears relatively insensitive to it and to changes in the circulating levels of natural ACTH between about 90 and 130 days of gestation (Alexander et al., 1973b; Liggins et al., 1973; Alexander et al., 1974a; Jones, 1975; Jones et al., 1975; Wintour et al., 1975), and there is no apparent increase in plasma ACTH levels before the prepartum rise in fetal plasma corticosteroid concentrations (Rees, Jack, Thomas and Nathanielsz, 1975). In addition, Jones (1975) has identified several different types of natural ACTH in the plasma of fetal sheep, the relationship and relative activities of which have still to be established. An increasing reponsiveness of the adrenal glands to levels of circulating ACTH in late pregnancy would explain why plasma corticosteroid concentrations increase when ACTH concentrations do not change and could explain the apparent lack of negative feedback of corticosteroids on the pituitary during this period. Liggins et al., (1973) infused cortisol (25 mg/24 hr) into the ovine fetal vasculature at a rate comparable to the maximum production rate of cortisol at delivery (20-25 mg/24 hr; Nathanielsz et al., 1972) and obtained premature parturition. Bosc (1972) found

dexamethasone given i.m. to the ewe induced premature parturition in control but not in hypophysectomised sheep fetuses. On the other hand, parturition did not occur when Liggins (1968) infused cortisol (e.g. 100 mg/24 hr) into the ewe.

Discrepancies between the relative potencies of cortisol and dexamethansone and the amounts given to the fetus which are necessary to cause premature birth (Liggins, 1969a) question the action of dexamethasone. Finally, premature separation of fetal and maternal tissues in particular areas of the placental cotyledons occurs during parturition following continuous cortisol infusions (Jack, Nathanielsz, Thomas and Steven, 1975).

Changes in the concentrations of other hormones in fetal plasma have also been observed and although their role in parturition is uncertain, their involvement cannot be discounted. The concentrations of noradrenaline in maternal and fetal plasma of sheep increase during the last 72 hrs before birth to reach a peak at or just before birth itself (Phillippo, Lawrence and Mellor, 1974), as do fetal plasma concentrations of arginine vesopressin (Alexander et al., 1974b). In ovine fetuses plasma growth hormone (GH) concentrations decrease before birth (Bassett, Thorburn and Wallace, 1970) and plasma thyroxine concentrations either decrease or remain relatively unchanged until labour when a marked rise in concentration occurs (Nathanielsz, Comline, Silver and Thomas, 1973a; Thorburn and Hopkins, 1973; Mellor, Matheson, Small and Wright, 1976). Finally, in addition to hormones, maternal and fetal plasma glucose concentrations are elevated during labour and fetal plasma lactate concentrations may increase some hours

before birth. However, fetal blood PO_2, PCO_2 and pH remain relatively stable until delivery (Comline and Silver, 1972).

It is impossible to ascertain from the present data whether or not fetal plasma corticosteroids play the major role in initiating parturition. It is quite conceivable that the fetal trigger which terminates pregnancy comes via the hypothalamo-pituitary axis to endocrine systems as a whole and depends upon the integrity of many endocrine glands in addition to the adrenals.

Several suggestions have been made about the initial stimulus to parturition. Nathanielsz et al. (1972) suggest maturation of tissues such as the lungs and central nervous system, Mellor and Slater (1974) suggest fetal compression, as a result of fetal growth and a reduction in amniotic fluid volume, and Hopkins and Thorburn (1971) and Thorburn et al. (1972) pointed to maturation of hypothalamic thermal receptors and an increasing fetal 'awareness' of mild thermal stress.

The initial stimulus to parturition in sheep and goats may well depend upon several factors which may act simultaneously. The spread of gestational ages at parturition is so narrow that the timing of birth, which is critical for survival of the young, is not likely to depend solely on one stimulus. In fact, the fetal stimulus to parturition may be different in different conceptuses. For example, compression of the fetus, and the capacity of the uterus may be more important in twin pregnancies, whereas signals from maturing tissues may provide the major stimulus in single pregnancies. In goats at

least, a maternal stimulus to parturition in the absence of, or reinforcing a fetal stimulus cannot be ruled out, for anything triggering luteal regression whether it be fetal or maternal in origin is sufficient to cause parturition regardless of the stage of fetal development.

The period immediately after birth is probably the most critical time for the newborn. Major changes in circulation and metabolism take place almost immediately after delivery with the replacement of the functioning placenta by pulmonary gaseous exchange and gastro-intestinal absorption of metabolites (e.g. Dawes, 1968; Edwards, 1970; Hull, 1975; Shelley et al., 1975). Many observations have been made on the lamb during its first few weeks of life (e.g. studies by G.Alexander and colleagues). However, except for studies by Edwards (1970) and Comline et al., (1974) on calves and one by Comline and Silver (1972) on lambs little detailed information is available on changes in plasma composition immediately after birth. Apart from a limited study of brown adipose tissue deposition (Thompson and Jenkinson, 1970) the physiology of the newborn goat has apparently received little attention.

An obvious and perhaps necessary extension of the 'chronic' catheterisation studies of the fetus is to examine the neonate from which intrauterine data have been obtained. A detailed study of behaviour and changes in plasma composition in 17 lambs and three kids during the first 7-18 hr of extrauterine life has been undertaken.

Surgical catheterisation is likely to affect plasma composition and cannot be accomplished quickly after birth, while venepuncture may also be stressful and is difficult in the newborn. Catheters inserted directly into the fetus during pregnancy can affect development (Alexander et al., 1973b; Mellor and Matheson, 1975) and may pull out during delivery (Comline and Silver, 1972), while those placed in the placental circulation cannot be used after birth. Since none of these usual blood sampling techniques appeared satisfactory, a method was used which involved inserting catheters into the aorta or vena cava via the cut umbilical vessels of the newborn at delivery. This enabled arterial or venous blood to be taken almost immediately after birth and for several hours thereafter, with minimal stress to the lamb or kid.

8.2 EXPERIMENTAL METHODS

8.2.1 Animals

Seventeen lambs (birth weights 2.5-4.8 kg) from 12 ewes and three kids (birth weights 2.6-2.7 kg) from three goats were used and were kept in a well ventilated animal house at a daily air temperature of 4-17°C.

8.2.2 Catheters

An umbilical vein of 12 lambs and an umbilical artery of five lambs and three kids were catheterised and the first blood sample taken 1½-6 min after birth as described in Chapter Two. The catheters were sampled at intervals over 7-18 hr, and were then removed.

8.2.3 Measurements

Behaviour. All animals were observed continuously until sucking occurred (45min - 8 hr after birth) and thereafter at 10-20 min intervals. The time taken for the newborn to stand, show teat-seeking activity and suck for the first time after birth without assistance were recorded. Teat-seeking activity was said to occur when lambs or kids showed upward thrusting movements of their head or neck, usually against some part of the ewe or pen and usually accompanied by tail wagging and nibbling movements of the lips. Maternal attention (e.g. licking and nudging) and any signs of mismothering were also noted.

Body temperature. Rectal temperature of the newborn was measured at five minute to three hour intervals.

Blood sampling. The total time taken from restraining the neonate for sampling to releasing it after sampling never took more than two minutes and removal of blood samples from the catheters took less than 15 seconds. Where possible, samples were taken at 2,5,10, 15,30,45,60 and 90 min and 2,4,7,11,15 and 18 hr after birth. Usually not more than five samples were taken within the first hour of life.

Analyses. Blood gas and pH measurements were determined in samples from nine lambs and one kid only. Other analyses were carried out on all samples.

Results from arterial and venous blood samples were pooled

except where there were obvious differences and where relevant are expressed as mean ±SD. Except where stated all times given in the results are taken from the time of birth in each individual (time 0).

8.3 RESULTS

8.3.1 Lambs

a. Behaviour

Two ewes had difficulty at delivery and help was given. One lamb (313) was a breech presentation and another presented upside down. All lambs shivered between 5 and 50 min, stood without assistance at 8 to 30 min, and showed strong teat-seeking activity 12-48 min after birth. During initial teat-seeking activity lambs did not go directly to the udder but tended to remain near the front of the ewe. Twelve of 17 lambs found the teats and obtained colostrum within 90 min of birth while three others took 3½,5 and 7 hr. The time taken to the first feed appeared to depend upon the position of the teats and the persistence of the lambs. A set of twins had failed to suck after 8 hr, despite normal attention from the ewe, because the position of the teats on the udder made them relatively inaccessible to the lambs. After 6 hr, teat-seeking and general activity in these two lambs had decreased markedly. This is consistent with the behaviour of lambs prevented from sucking by a cover over the udder (Alexander and Williams, 1966). With assistance, these two lambs obtained milk and thereafter showed behaviour comparable to other lambs of a similar age although some assistance in sucking (restraining the ewe) was necessary for the subsequent 6 hrs until the udder became less distended. In contrast Clun Forest and Suffolk x Masham lambs kept as a group took

approximately 13-14 min to stand but obtained milk more quickly, usually sucking about 30 min after delivery (Bareham, 1976).

After the first feed, teat-seeking activity decreased and all lambs generally lay down, and stood again at intervals when stimulated by the ewe or on their own initiative to feed. Blood sampling was carried out within reach of the ewe and did not appear to disturb this pattern of behaviour as the behaviour of catheterised lambs, uncatheterised littermates and other lambs was indistinguishable.

The ewes appeared to accept the presence of the experimenter, although some remained alert and defensive when their lambs were handled. Providing the protective bandage around the catheter was soaked with amniotic fluid at birth the ewe showed no interest in the catheters and no ewe in this study showed signs of rejecting her offspring. The degree of maternal attention given was similar to that generally observed with uncatheterised lambs and to that described by Alexander (1960) and Fraser (1968).

All lambs survived until weaning and showed normal behaviour and growth rates during the first month (range 0.5-1.76 kg/week).

b. Body temperature

Rectal temperature of the lambs within 10 min of birth was $40.5 \pm 0.3^{\circ}C$. It then decreased by 0 to $1.2^{\circ}C$ during the following 5 min but returned to previous values ($40.2 \pm 0.2^{\circ}C$) within 20 min of birth even though the coat was still wet. Thereafter body temperature

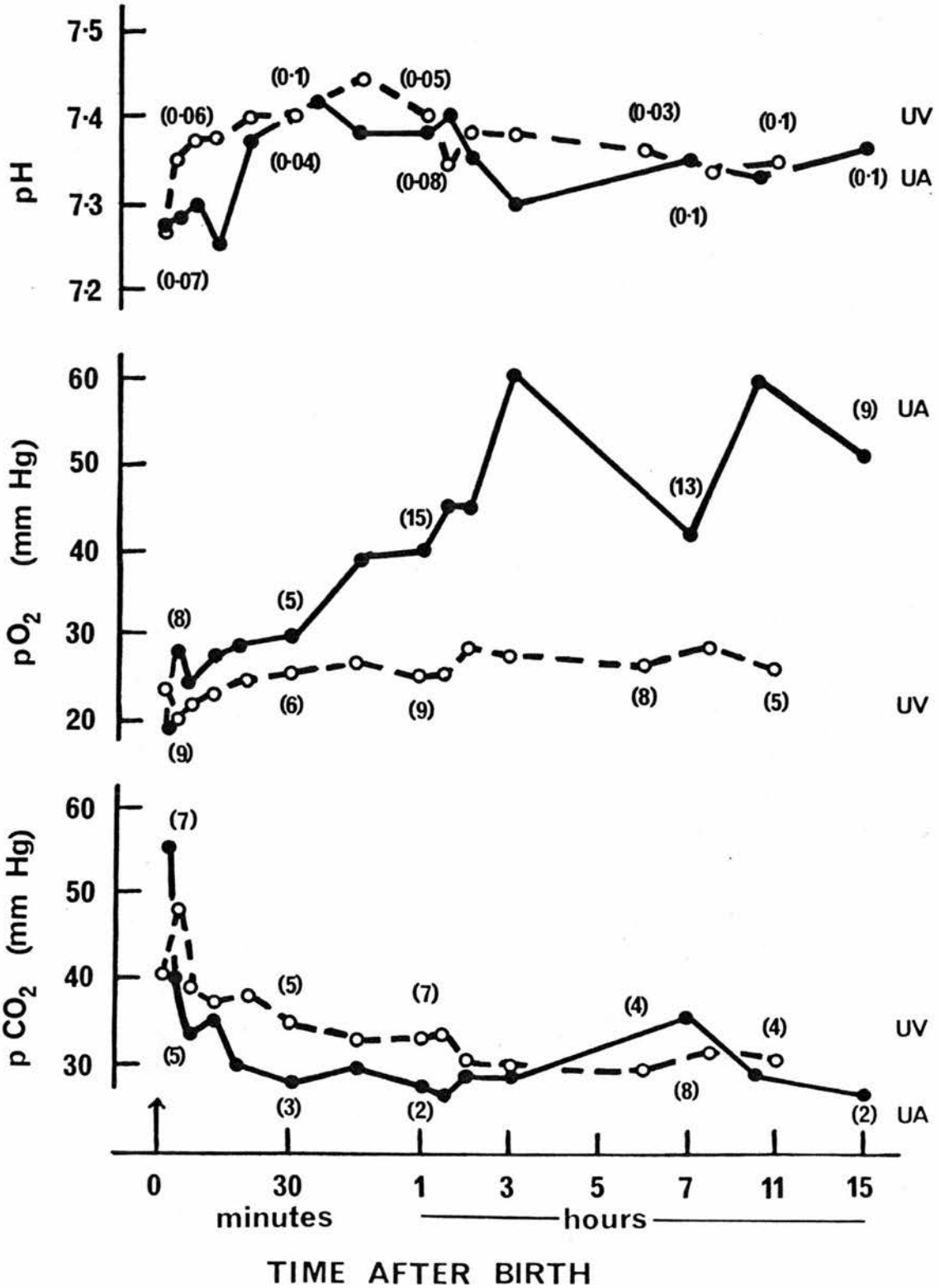

Figure 8:1

Changes in mean pH, PO_2 and PCO_2 values in arterial blood (●) from four lambs and venous blood (○) from five lambs during their first 11-15 hours after birth (arrow). Standard deviations are given in brackets.

remained constant. This is consistent with the findings of Sykes, Griffiths and Slee (1976) that Scottish Blackface lambs have a higher tolerance to cold during the first day of life than other breeds of sheep.

c. PO_2, PCO_2 and pH.

Mean ($\pm$SD) values for arterial and venous samples from different animals are given in Fig.8:1. PO_2 increased markedly in arterial blood between 1 and 3 hr and thereafter showed fluctuations of up to 30 mm Hg between samples. PO_2 in venous blood showed some fluctuations during the first hour but then remained relatively constant. PCO_2 had reached minimum values by 20 min in arterial blood and by about 60 min in venous blood. PCO_2 tended to be higher in venous than in arterial samples but not significantly so. Associated with these changes was a marked increase in pH during the first hour of life in all animals. Mean arterial and venous PCO_2 (29 and 34 mm Hg) values 60 minutes after birth were similar to those found in adult sheep (Comline, Silver and Silver, 1965; Hales and Webster, 1967; Parker and Purves, 1967; Comline and Silver, 1970; Mitchell and Williams, 1975) but arterial PO_2 (40 mm Hg) was lower. Arterial PO_2 values in lambs (63 mm Hg at two weeks of age) do not reach adult values (88 mm Hg) until 20 weeks after birth although values of 80 mm Hg are found at eight weeks of age (Mitchell and Williams, 1975).

d. Lactate

Individual variation in plasma lactate concentrations during the first 5 min after birth was large (range 600-1140 mg/l).

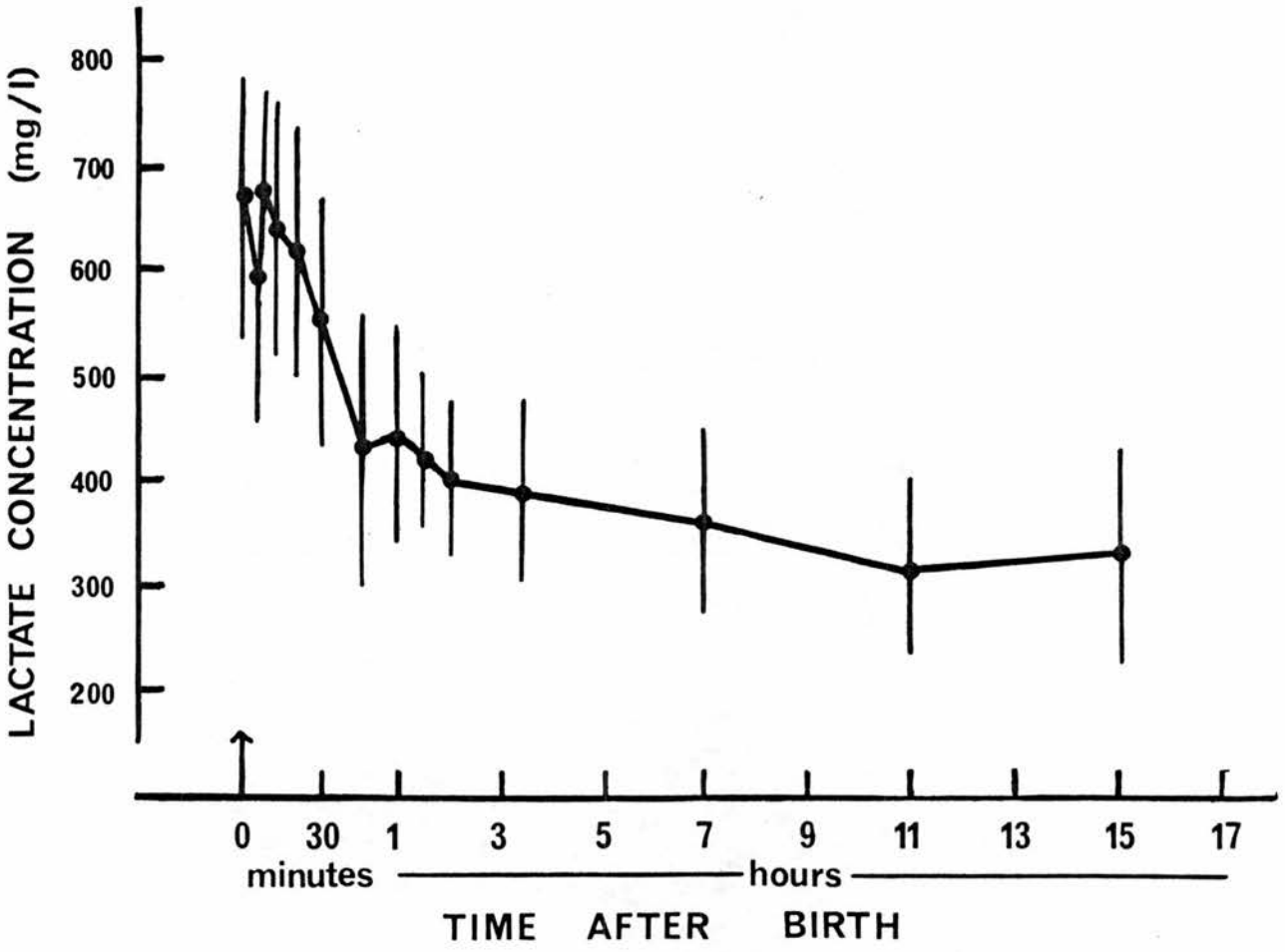

Figure 8:2

Changes in mean (±SD) concentrations of lactate in plasma from a total of nine lambs during their first 16 hours after birth.

Concentrations remained elevated between 15 and 30 min in all animals and thereafter decreased to reach relatively stable values (250-400 mg/l) at 9 to 15 hr. Plasma lactate concentrations in the nine animals, in which blood pH was also measured, decreased slowly after birth (Fig.8:2) compared to a more rapid rise in pH (Fig.8:1). Similar changes were observed in lambs by Comline and Silver (1972).

e. PCV

PCV was not significantly different in arterial and venous blood samples. However, widely differing basal PCV values were found in individual lambs at birth (range 29-50%). Changes in PCV (1-3%) were not consistent until 1-3 hr after the lambs had sucked, when a significant ($P < 0.05$) decrease in PCV had occurred (Table 8:1). This decrease continued as the lambs continued feeding and reached minimum values 6-10 hr after the first feed.

f. Thyroxine

The concentrations of T_4 in plasma varied considerably between animals with concentrations of 80-200 ng/ml being observed between 1½ and 6 min. Changes of up to 40 ng/ml in plasma T_4 concentrations occurred in individual lambs from first sample to first suck. The pattern of these T_4 changes was not consistent within the group, and did not appear to be related to initial plasma T_4 concentrations. The most marked changes occurred when the lambs obtained milk for the first time. T_4 concentrations in all lambs then decreased steadily during the 1-3 hr after sucking as shown by the mean values given in Table 8:1.

TABLE 8:1

Mean ($\pm$SD) values for packed cell volume (PCV) and the concentrations of glucose and thyroxine (T_4) in plasma from a total of 16 newborn lambs before and after first feed. The significance of differences in values after sucking from those observed before first suck are given.

Time	Glucose mg/l	PCV %	T_4 ng/ml
1 hr before sucking	407$\pm$140 (16)	37.5$\pm$4 (16)	147$\pm$35 (16)
----------FIRST FEED----------			
1 hr after sucking	940$\pm$400 (16)***	37.8$\pm$3 (16)^{ns}	140$\pm$33 (16)^{ns}
1-3hr after	960$\pm$320 (16)***	33.9$\pm$4 (16)*	135$\pm$39 (16)^{ns}
3-6hr after	1010$\pm$280 (16)***	31.8$\pm$2 (16)***	114$\pm$30 (16)**
7-10hr after	1200$\pm$420 (16)***	29.0$\pm$2 (16)***	102$\pm$34 (16)***
14-17hr after	1100$\pm$180 (5)***	30.3$\pm$1 (5)***	108$\pm$40 (5)*

^{ns} not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

The number of observations are given in brackets.

g. Glucose

Plasma concentrations in lambs immediately after birth varied from 180-800 mg/l. Thereafter, with one exception, concentrations remained relatively constant or decreased until first suck, after which a significant ($P<0.001$) increase in concentration occurred in all animals (Table 8:1). The exception was a lamb (313) which had a difficult birth and was delivered manually. Plasma glucose concentrations in this lamb increased from 520 mg/l at 2 min and reached 1200 mg/l at 30 min. By 90 min the plasma concentration had returned to about 700 mg/l where it remained until the lamb obtained milk at 3½ hr. The plasma concentration had increased to 1250 mg/l in the 4 hr sample.

h. Fructose

Regardless of the changes in other plasma constituents the plasma concentration of fructose decreased exponentially in all lambs during the sampling period (7-18 hr: Fig.8:3). Using a multiple regression program (Multireg, ICL 475 computer) the closeness of fit to an exponential curve for the decrease in plasma fructose over time was described for each lamb. Correlation coefficients of 0.945-0.999 ($n = 8-15$) were found. Therefore, the rate of loss of fructose from the plasma was directly proportional to its concentration.

In the steady state ovine fetus the concentration of fructose in the erythrocytes is about 78% of that in plasma (Hitchcock, 1949; Widdas, 1955). Fetal erythrocytes are freely permeable to fructose (Widdas, 1955) and after birth when plasma fructose concentration is decreasing red cell fructose concentration will be greater than or

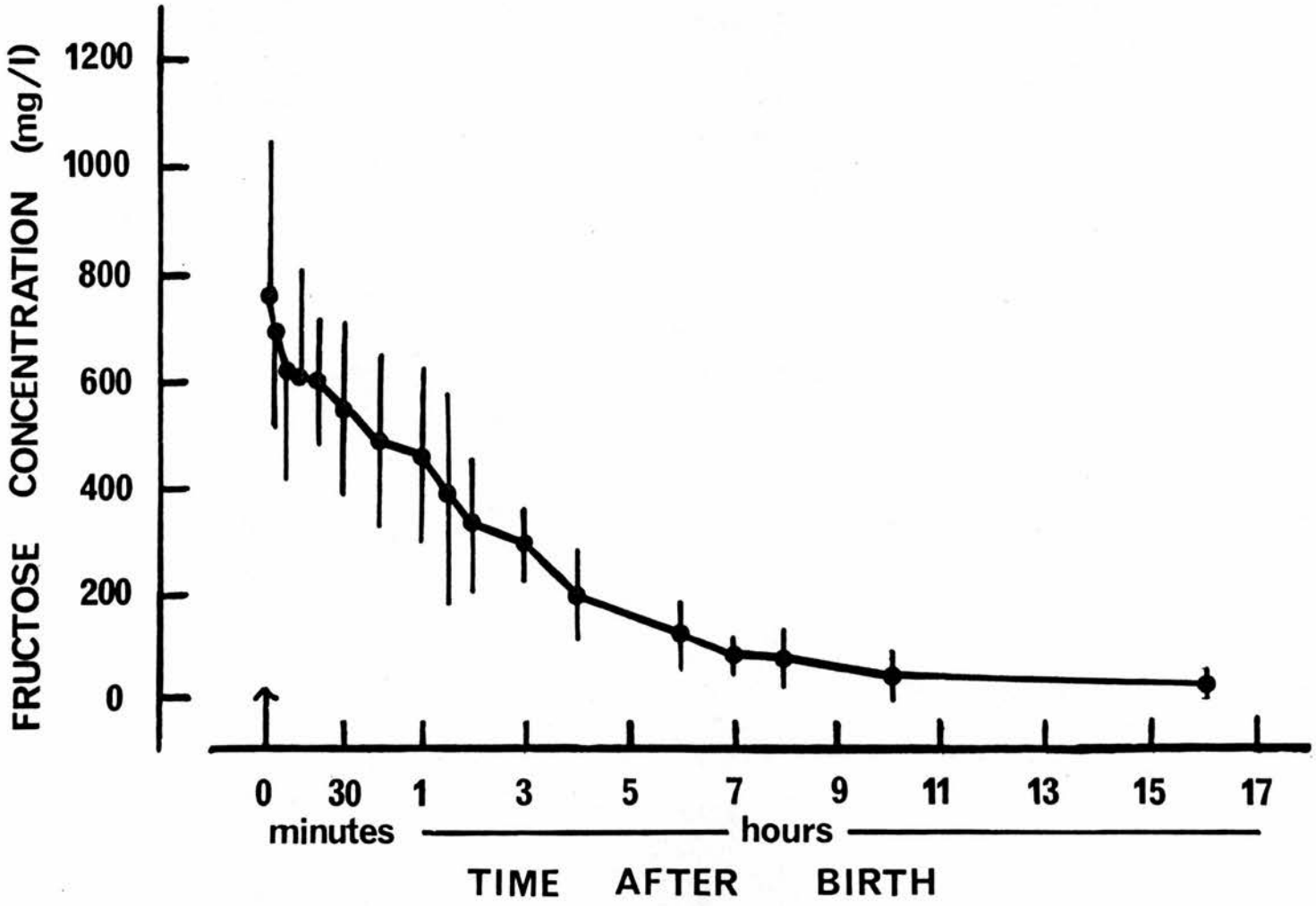

Figure 8:3

Changes in mean (±SD) concentrations of fructose in plasma from a total of 16 lambs during their first 16 hours after birth.

equal to that of plasma. An assumption that blood volume represents total blood water (and not water plus the cellular fraction) with respect to fructose allows this potentially greater concentration of intracellular fluid fructose to be at least partly compensated for, so that at birth (time 0) it may be assumed that the concentration of fructose in whole blood is approximately equal to its concentration in plasma. Since erythrocytes are freely permeable to fructose (Widdas, 1955), it is reasonable to assume that the rate of decline in fructose concentrations of whole blood and plasma will be the same. Accordingly the rate of loss of fructose from a unit volume of blood (plasma) at birth was derived from the slope of each exponential equation at time 0. Taking the blood volume of the lamb at birth as 111 ml/kg body weight (Creasy, Drost, Green and Morris, 1970) an estimate of the total rate of loss of fructose from blood in these lambs can be obtained. Since the mean ($\pm$SD) decrease in plasma concentration of fructose at time 0 (slope) was 165 ± 90 mg/l/hr, the estimated total rate of loss of fructose from blood was 66.8 ± 38.2 mg/hr.

In addition, the clearance rate of fructose, i.e. the volume of plasma that would in theory be completely cleared of fructose per unit time was calculated for each lamb using the exponential curve data.

$$\text{Clearance} = \frac{\text{rate of loss at time 0}}{\text{plasma concentration at time 0}}$$

Rate of clearance of fructose from plasma at birth in the 17 lambs was 26.5 ± 8.1 ml/hr.

i. Corticosteroids

The concentration of corticosteroids in plasma increased

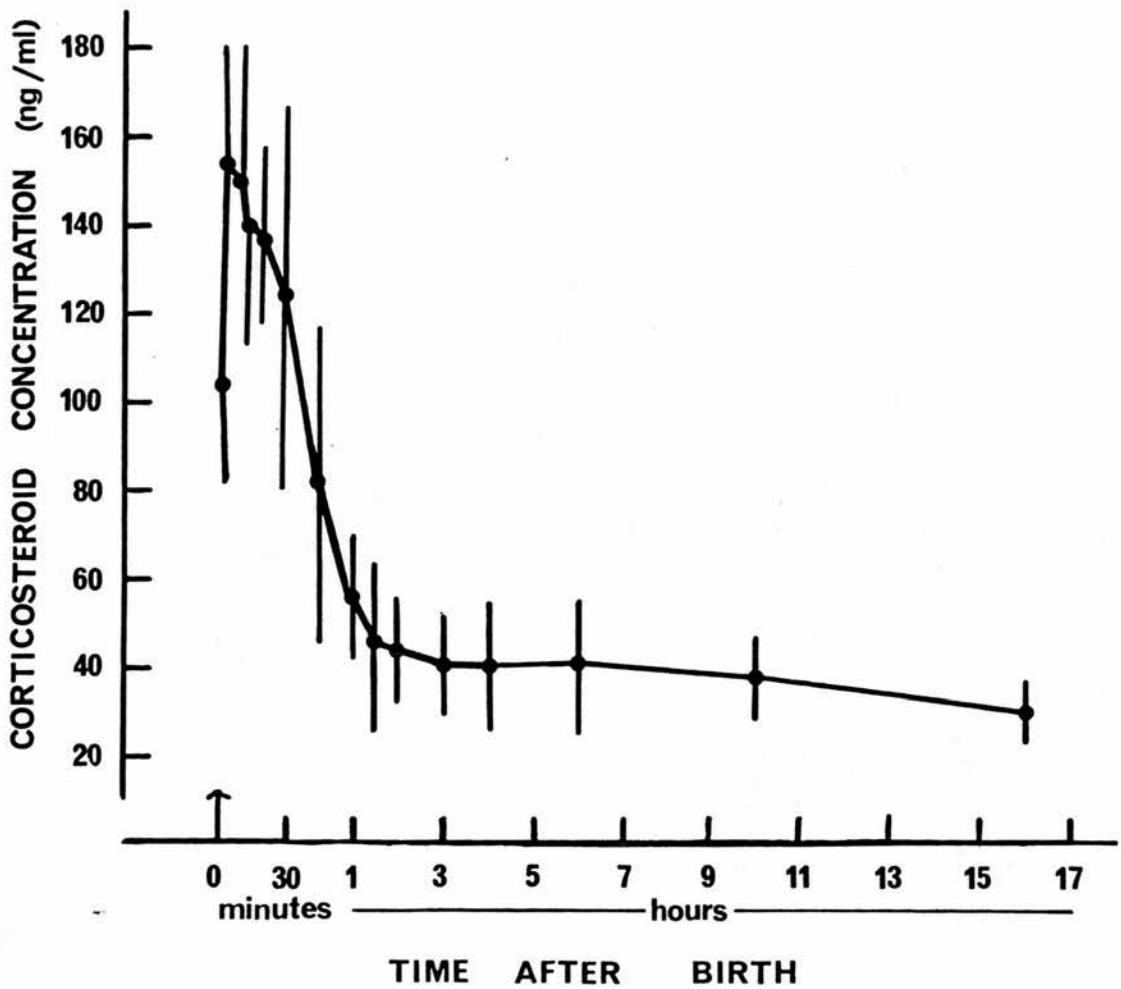

Figure 8:4

Changes in mean (±SD) concentrations of corticosteroids in plasma from a total of 16 lambs during their first 16 hours after birth.

significantly ($P < 0.01$) during the first 5 min after birth, and thereafter began to decrease, the fall becoming significant ($P < 0.001$) at 45 min. After 60 min the corticosteroid concentrations decreased more slowly and reached about 20 ng/ml at 16 hr (Fig.8:4).

8.3.2 Plasma composition after haemorrhage

Before one of the lambs had sucked, the ewe damaged the tap on an arterial catheter causing a haemorrhage. A decrease in PCV (from 50 to 36%) and pH (from 7.32 to 7.25) and an increase in PO_2 (from 33 to 53 mm Hg) and lactate (from 300 to 1130 mg/l), glucose (from 600 to 1680 mg/l) and corticosteroid (from 62 to 219 ng/ml) concentrations occurred. No changes in PCO_2, T_4 or fructose concentrations were observed. Half an hour later the lamb sucked, and in a sample taken 3 hr after haemorrhage only plasma lactate (940 mg/l) and corticosteroid (51 ng/ml) concentrations were greater than values found in lambs that did not haemorrhage. Seven hours after haemorrhage plasma lactate and corticosteroid concentrations had decreased to 400 mg/l and 28 ng/ml, respectively, and were then similar to those observed in other lambs at the same postnatal age.

The rapid increases in plasma corticosteroid concentrations in response to haemorrhage in this lamb contrast markedly with the relatively small changes that were observed in acute fetal preparations after haemorrhage despite large increases in fetal plasma ACTH levels (Alexander et al., 1974a). This is in keeping with the apparently greater responsiveness of the adrenal gland to circulating ACTH after birth than during most of fetal life (Boddy, Jones, Mantell, Ratcliffe and Robinson, 1974). Apart from cortico-

steroid concentrations, the changes in plasma composition after haemorrhage were similar to those observed in the sheep fetus by Alexander et al., (1974a).

8.3.3 Plasma composition after removal from the ewe and with associated mild cold exposure

A set of twins were removed from their mother at 52 min while still wet and were placed in a room at 4°C for 2 hr. Body temperatures remained constant and apart from corticosteroid concentrations no significant changes in plasma composition occurred. Corticosteroid concentrations during the first hour (63 ± 12 ng/ml (4)) increased to values of 106 ± 25 ng/ml (4) during the second hour in the cold ($P < 0.05$). The concentrations decreased to 50 ± 3 ng/ml (2) within 4 hr of being returned to the ewe ($P < 0.05$).

8.3.4 Kids

a. Behaviour and body temperature

Since there was a close association between behaviour and body temperature the two are reported together. No difficulties occurred during parturition in any of the goats. Kid A was active from birth, was standing at 15 min, teat-seeking at 25 min and obtained colostrum at 50 min. Body temperature remained constant (38.5-38.7°C). Kid B was relatively inactive after birth. It made no attempt to stand until 35 min, was standing at 60 min, showed teat-seeking activity at 2 hr and sucked for the first time at $3\frac{1}{2}$ hr. Body temperature in this kid decreased from 38.5°C at 15 min to 36.2°C at 90 min and then increased slowly to 38.5°C at 5 hr. Both kids showed signs of shivering for 2 hr (A) and 5 hr (B) after birth. Behaviour after sucking was similar to that of the lambs and

maternal attentions seemed normal. Kid C was inactive at birth and remained lying down, made no attempts to stand and stopped shivering as it became progressively weaker. Sixty minutes after birth the kid's body temperature was 33°C and it was moved with the doe to a warm room (20°C) where the kid was dried and warmed with a fan-heater. After 2 hr it began to shiver and was given 10 ml colostrum; after 3 hr its body temperature levelled out at 38.5°C; between 3 and 4 hr activity increased, it attempted to move when licked strongly by the doe, showed weak teat-seeking activity and obtained some milk when held to the udder; after 6 hr it could stand unaided and began teat-seeking near the udder; between 6 and 10 hr (7-11hr after birth) it sucked from the udder. The kid was kept in the warm room for 24 hr by which time it showed activity similar to other kids, and was returned to the original pen with the doe. Apart from a lack of interest during the first 45 min after birth, the doe appeared to accept the kid and showed normal behaviour towards it.

b. Plasma composition

PO_2, PCO_2 and pH changes in blood (arterial) were only measured in one kid (B) and were similar to those observed in the lambs. An increase in pH (from 7.18 to 7.40) and PO_2 (from 49 to 65 mm Hg) and a decrease in PCO_2 (from 42 to 30 mm Hg) occurred during the first 30 min after birth and thereafter pH and PCO_2 values remained relatively constant while PO_2 fluctuated.

PCV in all three kids remained constant before sucking (A - 44$\pm$0.8% (4), B - 36.5$\pm$1% (9) and C - 37$\pm$0.9 (8). Within 4 hr of sucking PCV decreased to 26-31%.

Fructose concentrations in plasma in all three kids (concentrations at 1½ to 4 min were 390 (A), 1000 (B) and 420 (C) mg/l) decreased exponentially after birth. Estimated total rate of loss of fructose from blood at birth, calculated as above, was 40.1, 45.5 and 37.0 mg/hr and fructose clearance rate from plasma was 36.8, 14.9 and 29.2 ml/hr in kids A,B and C, respectively.

Glucose, corticosteroid, lactate and T_4 concentrations. Kid A (active). Plasma glucose (510 ± 50 mg/l (4)), lactate (815 ± 90 mg/l (4)) and T_4 (150 ± 8 ng/ml (4)) concentrations during the first 30 min after birth were within the range observed in lambs and showed similar changes from birth and after sucking to those observed in lambs. Between 0 and 6 min the plasma corticosteroid concentrations increased from 242 to 262 ng/ml and then decreased to 46 ng/ml at 9 hr.

Kid B (less active). During the first 10 min after birth plasma glucose concentrations were about 610 mg/l and corticosteroid concentrations decreased from 290 to 172 ng/ml. However, the decrease in body temperature during the first 90 min after birth was associated with an increase in glucose and corticosteroid concentrations which both remained high (1300-1400 mg/l and 240-250 ng/ml, respectively) until body temperature returned to normal at 5 hr. An hour later, glucose and corticosteroid concentrations were 800 mg/l and 70 ng/ml, respectively. During the first 4 hr after birth lactate concentrations remained above 1220 mg/l reaching highest values (1500-2000 mg/l) when body temperature was at its lowest. T_4 concentrations (110 ± 7 ng/ml (9)) remained relatively

constant during the change in body temperature but decreased after sucking to 90 ng/ml 3 hr after the first feed.

Kid C (inactive). Body temperature reached a minimum value (33°C) at 60 min then increased to 38.5°C at 4 hr. Unlike kid B the associated increase in glucose concentration was small (570 to 760 mg/l) and occurred slowly during the first 90 min after birth. Corticosteroid and lactate concentrations increased from 281 ng/ml and 1320 mg/l to reach 335 ng/ml and 2000 mg/l, respectively, at 90 min when body temperature was at its lowest. When body temperature increased, glucose concentrations decreased to 240 mg/l at 4 hr and thereafter gradually increased as milk was ingested to 670 mg/l at 11 hr. Corticosteroid and lactate concentrations remained greater than 218 ng/ml and 1490 mg/l, respectively, until 4 hr then decreased to 112 ng/ml and 1040 mg/l, respectively, at 11 hr. T_4 concentrations were low and remained low (54 ± 3 ng/ml (8)) until 4 hr (when body temperature had returned to normal) when an increase to 80 ng/ml occurred. T_4 concentrations then remained between 60-73 ng/ml as milk intake increased.

8.4 DISCUSSION

With practice catheterisation could be carried out and a blood sample obtained within two minutes of birth so the technique provides a simple, quick method of obtaining frequent blood samples from the neonate. From the apparently normal behaviour of the animals it was clear that the catheterisation procedure itself and sampling did not unduly affect the neonate or disturb the general pattern of behaviour observed in mother and offspring after birth.

The circulation of the fetus and the changes associated with loss of the placenta at birth are now relatively well known and, as they have been outlined in detail by Dawes (1968) are only briefly refered to here. In the fetus, the two sides of the heart work in parallel to pump blood from the great veins to the aorta by way of the foramen ovale, between the inferior vena cava and the left atrium, and the ductus arteriosus, between the pulmonary artery and aorta. Blood flow through the fetal lungs, which have a high vascular resistance, is small. From the aorta blood passes to the tissues of the fetus and via the umbilical arteries to the placenta where oxygenation occurs. From the common umbilical vein the oxygenated blood flows into the liver and through it (in the ductus venosus) to the right and left atria where partial mixing of oxygenated and deoxygenated blood occurs.

At birth, the umbilical cord is severed and the newborn animal has then to function independently of the mother, whose only contributions to the well-being of the newborn are intermittent provision of passive immunity, food, warmth, protection and shelter. Pulmonary respiration is initiated almost immediately as inflation of the lungs occurs and pulmonary blood flow increases. The blood pressure changes which occur in the heart and major vessels are associated with rupture of the umbilical cord, and these plus the increase in pulmonary blood flow at birth (Dawes, 1968) result in the gradual closure of the foramen ovale. The ductus arteriosus constricts rapidly at first so that its lumen is considerably narrower within 10-30 min after respiration has begun in the lamb (Dawes, 1961). However, because of the rise in systemic vascular

resistance and the fall in pulmonary vascular resistance the direction of blood flow through the ductus may be reversed so that blood flows from the aorta into the pulmonary artery (left to right (L-R) shunt). Thereafter, the ductus constricts more slowly and within days or weeks anatomical closure of the lumen takes place. The ductus venosus of the lamb apparently closes at or soon after birth (Dawes, 1968). The presence of umbilical venous catheters would have prevented closure of the ductus venosus although effectively blocking it. The position of the catheters was not examined, however, from the distances they were placed in the neonatal umbilical vessels (Section 2.4.3) it is estimated that in most cases the tips of the catheters were in either the posterior aorta or vena cava near the heart.

The low PO_2 and pH and high PCO_2 observed at birth in the present lambs were also found by Comline and Silver (1972) in lambs and Comline et al. (1974) in calves. Hypoxia and hypercapnea probably resulted from compression and occlusion of the umbilical cord during labour and at delivery before breathing was established. The onset of normal rhythmical breathing was associated with a rapid rise in arterial PO_2 and fall in PCO_2. The increase in PO_2 apparently triggers off muscular closure of the ductus arteriosus (Dawes, 1968; Fay, 1973). Low blood pH at birth appears to result from respiratory acidosis as pH values increased when PCO_2 decreased. Right to left (R-L) shunts through the foramen ovale occur in a high proportion of lambs less than 12 hr old. R-L shunts through the ductus arteriosus have not been detected and L-R shunts appear to be relatively unimportant (Stahlman, Merrill and Le Quire, 1962; Alexander and

Williams, 1970). Marked fluctuations in arterial PO_2 that occurred in the present lambs after birth can be accounted for by a R-L shunt through the foramen ovale which would decrease the PO_2 of aortic blood. Alternatively, the fluctuations may have resulted from variations in intrapulmonary or ductal shunts. Comline and Silver (1972) did not observe similar fluctuations in lambs in which collection of blood through hindlimb arterial catheters was relatively slow, but the present method allowed rapid collection and therefore the detection of relatively rapid and transient change.

Absolute values and changes in the concentrations of plasma metabolites before sucking will be influenced largely by trauma at birth, the physiological capabilities of the newborn and the environment to which it is exposed at delivery. These effects will be different in each animal and probably account for the extremely wide range of glucose and lactate concentrations observed in the lambs immediately after birth. Rapid mobilisation of glycogen reserves immediately after birth (Shelley, 1961, 1964) may be responsible for the maintenance of relatively constant plasma glucose concentrations in unfed lambs in the present and other studies (e.g. Alexander and Mills, 1968; Edwards, 1970) and for the increase in glucose concentrations observed in unfed calves (Edwards, 1970; Edwards and Silver, 1970). It has been suggested that glycogenolysis is due to catecholamine secretion by the adrenal medulla (Shelley, 1960, 1964). However, stimulation of the hepatic sympathetic system, at least in newborn calves, appears to be a more likely mechanism (Edwards and Silver, 1970). Widespread stimulation of the sympathetic system at birth may be the stimulus to brown fat

metabolism (Hull, 1966) and the cause of the rapid rise in plasma FFA concentrations which occurs within two hours of birth in lambs (Van Duyne, Parker, Havel and Holm, 1960; Alexander and Mills, 1968; Comline and Silver, 1972). Although FFA values were not measured in this study the generally higher glucose and lactate values in plasma are consistent with increased sympathetic activity at birth. The marked rise in glucose concentrations seen in one lamb after a difficult delivery was also observed by Alexander and Mills (1968) under similar circumstances.

In dogs, a change from peripheral muscular vascoconstriction to vasodilatation occurred after pulmonary expansion (Daly and Robinson, 1968). If a similar change occurs in lambs, then the lactate which has accumulated in peripheral tissues during hypoxia may be released into the circulation and at least partly account for the high plasma lactate levels after birth. The slow decline in plasma lactate concentrations in the lambs, and in calves (Edwards, 1970) after respiratory acidosis had ceased suggests one or both of the following: anaerobic glycolysis continues at a gradually decreasing rate after birth or metabolism of lactate by the liver is reduced during the first day of life (Andrews, Britton, Huggett and Nixon, 1960).

The presence of the oesophageal groove in the neonatal ruminant usually ensures that ingested milk passes directly to the abomasum and intestines where the ability to absorb macromolecules is present for about 60 hr after birth (Hardy, 1970). The marked increase in plasma glucose concentrations in lamb plasma within 1 hr of sucking is consistent with the rapid absorption of glucose and gluconeogenic

substances from the neonatal gut (Hill et al., 1970) and is associated with an increase in plasma insulin concentrations in lambs less than 24 hr old (Bassett and Alexander, 1971). In older lambs (1-3 months) a transient increase in insulin and decrease in GH concentrations precedes changes associated with an increase in plasma glucose concentrations after sucking (Bassett, 1974a), but it is not known if such changes occur during the first day of life.

The amount of blood removed from each lamb would at most account for a fall of 3% in the PCV. Thus the observed decrease of 9% could not have been entirely due to sampling. The decrease may be partly explained by the transition from fetal to adult haemoglobin which is practically completed during the first 40 days after birth in sheep and goats (Breathnach, 1964; Huisman, Lewis, Blunt, Adams, Miller, Dozy and Boyd, 1969) and appears to be associated with a decrease in PCV during the first 20 days (Blunt and Huisman, 1975). As the greatest decrease in PCV occurred 1-3 hr after sucking, the decrease probably largely reflects an increase in plasma volume due to absorption of fluid from the gastro-intestinal lumen. The composition of milk (Altman and Dittmer, 1968) and the high rate of water turnover in newborn lambs (MacFarlane, 1975) favour fluid absorption from the gastro-intestinal tract rather than a shift of interstitial or intracellular fluid into the vascular compartment (MacFarlane, 1975). Up to 80% of the observed decrease in T_4 concentrations after birth could be accounted for by such an increase in plasma volume. The decrease in plasma T_4 concentrations, however, continues for the first 5-6 days of life in newborn lambs (Nathanielsz, 1970) and calves (Nathanielsz, 1969). In comparison,

tri-iodothyronine concentrations which are low in fetal plasma (Erenberg and Fisher, 1973; Nathanielsz, Silver and Comline, 1973b; Nathanielsz, 1975), increase at delivery and during the first few hours of neonatal life in lambs and calves (Nathanielsz, 1975).

The present study is the first detailed report of changes in plasma corticosteroids in individual lambs during the first day of life. The results show that the decrease in plasma corticosteroid concentrations occurs largely during the first 60 min of life and thereafter the changes are relatively small and slow (Fig.8:4). With less frequent sampling Paisey and Nathanielsz (1971) and Lopez and Phillips (1976) found that corticosteroid concentrations were elevated during the first day but decreased to relatively stable values about six days after birth in lambs and calves.

The significance of these and other hormone changes (e.g. a rapid decrease in plasma GH concentrations during the first 12 hr of life: Bassett and Alexander, 1971) is not fully understood but may be a cause or result of the striking enzyme changes which occur at or soon after birth (Walker, 1968).

It is not surprising that extensive changes occur during this period of adjustment from a continuous placental supply of metabolites to a relatively intermittent supply from ingested milk. Garel, Savajol and Barlet (1976) suggest that higher plasma calcitonin values found in newborn lambs relative to adult sheep may protect the young against nutritive hypercalcaemia after the change from continuous intrauterine to intermittent postnatal feeding. Marked

changes in mean blood composition (see above) and greater fluctuations in concentration which occur after birth, would invoke or require metabolic changes of similar magnitude.

The rapid decline in plasma fructose concentrations found during the first day in the present lambs also occurs in the calf (Edwards, 1970). Using the estimated rate of loss of fructose from the blood of the newborn lamb it is possible to calculate the rate of placental synthesis of fructose from glucose during late pregnancy. This can only be speculation as the following assumptions are made:

(1) Plasma fructose concentrations in individuals are similar at delivery and at the end of gestation before the prepartum hormone changes begin. This appears to be true in most lambs (Mellor, unpublished data).

(2) The fetus is in a steady state so that fructose influx into blood is equal to efflux.

(3) Entry of fructose into blood consists of two components; placental production and the amount being recycled as a result of swallowing and diffusion from the fetal fluids.

(4) Loss of fructose from blood is equal to the postpartum rate of loss (66.8 mg/hr), and largely results from renal excretion (Shelley and Dawes, 1962) although some fructose may be metabolised in the liver and other tissues.

(5) Recycling in swallowed amniotic fluid will be about 25.1 mg/hr, because about 334 ml of amniotic fluid is swallowed by the fetus each day in late gestation (Bradley and Mistretta, 1973), the concentration of fructose in amniotic fluid is about 175 mg/l (Mellor and Slater, 1973b) and fructose is absorbed from the

fetal gut (Wright and Nixon, 1961).

(6) Recycling from the fluids by diffusion directly into fetal blood is negligible.

Thus the difference between total influx (equal to the postpartum estimate of fructose loss; 66.8 mg/hr) and the amount being recycled (25.1 mg/hr) will approximate to the rate of placental fructose synthesis, which will be 41.7 mg/hr.

The mean body weight of the lambs in the present study was 3.59 kg so the estimate of placental synthesis of fructose = 11.6 mg/kg/hr. In comparison estimates of glucose uptake by the fetus in the last 10-15 days of pregnancy of 333-375 mg/kg/hr (Kronfeld, 1958; Comline and Silver, 1974), 276 mg/kg/hr (Crenshaw, 1970) and 184 mg/kg/hr (James, Raye, Gresham, Makowski, Meschia and Battaglia, 1972) have been reported. Therefore, the estimated placental synthesis of fructose would amount to 3-6% of the glucose requirement of the fetus in late pregnancy. This is consistent with the findings of Alexander et al. (1970a), Tsoulos, Colwill, Battaglia, Makowski and Meschia (1971), Setchell et al. (1972) and Burd, Jones, Simmons, Makowski, Meschia and Battaglia (1975).

The small number of kids makes comparison with the lambs difficult. However, the close similarity of the plasma composition of the active kid (kid A) and the lambs suggests that similar mechanisms are operating during the first 12 hr after birth. A striking difference between kids and lambs was the higher corticosteroid concentrations observed in all kids during the first day of life. Two kids showed marked decreases in body temperature within

90 min of birth. In contrast the Scottish Blackface lambs rapidly established homeothermy after delivery and two lambs, about 53 min old, showed no significant change in body temperature in response to a decrease in air temperature of 11°C. The increases in plasma glucose, lactate and corticosteroid concentrations during hypothermia in the two kids were similar to those observed in newborn lambs during cold stress (Alexander and Mills, 1968; Alexander, Mills and Scott, 1968b; Bassett and Alexander, 1971; Alexander, Bell and Hales, 1972b; Alexander et al., 1973c). Futhermore, the increases in plasma lactate and glucose concentrations during hypothermia in the two kids were consistent with an increase in the rate of glycolysis in muscle and the mobilisation of glucose from hepatic glycogen reserves. Increased lipogenesis of brown adipose tissue also occurs during cold exposure in lambs (Alexander et al., 1972b). Whether these changes are caused by stimulation of the adrenal medulla or direct sympathetic stimulation of the sites of substrate mobilisation or both is not clear but lambs and calves in a thermoneutral environment show similar changes when infused with catecholamines (e.g. Alexander et al., 1968b; Alexander, 1969, 1970a; Bassett and Alexander, 1971; Alexander, Bennett and Gemmell, 1975).

Kids were less able to maintain body temperature than lambs of an equal or lower birth weight and this must be due to a reduced ability to retain heat or to produce it or both. The short birth coat of kids would favour heat loss more than the thicker coat of the Blackface lamb when dry (Alexander, 1962). However, this effect may be minimal at birth since birth coats are wet after

delivery. The relative insulatory capacity of the tissues in the kids may also be lower. The maximum rate of heat production in response to cold will depend upon body reserves available for shivering and non-shivering thermogenesis. When fat and carbohydrate reserves are exhausted and have not been replenished by feeding then hypothermia can result (Alexander, 1970b). In two kids, however, the decrease in body temperature occurred soon after birth before body reserves were likely to have been severely depleted. This suggests that the capacity of these kids to mobilise and metabolise substrates immediately after birth was impaired.

Although total plasma T_4 concentration may not be a very accurate index of thyroid function (Nathanielsz, 1975) it is interesting to note that the kid (kid C) with the lowest concentration of T_4 in plasma at birth was the least active and was unable to maintain homeothermy. Alexander, Bell and Williams (1970b) found exogenous T_4 had no apparent effect on the thermogenic response to cold in the Merino lamb, although it altered basal metabolic rate. On the other hand, Irvine and Evans (1975) suggest that T_4 may indirectly stimulate thermogenesis in the foal by inhibiting catecholamine degradation in brown fat. In addition, enzymic reactions will be inhibited during severe hypothermia, and this will further reduce the newborn's capacity to produce and retain heat after delivery. However, further studies with more kids are required before any positive statements can be made regarding the importance of hormone differences to the relative abilities of individuals to maintain homeothermy and viability after birth.

Although the first few hours of life are critical to survival after birth, the success with which the transition from the intra-uterine to the extrauterine environment is accomplished will depend to a large extent upon the growth, development and maturation attained during pregnancy. Therefore, pregnancy is as important as the postpartum period in studies of the neonate and both periods should be considered together as a continuum rather than as two separate parts. The development of 'chronic' techniques has enabled both pre- and post-partum performance to be recorded in individual animals.

8.5 CONCLUSIONS AND COMMENTS

1. Non-surgical catheterisation of the aorta or vena cava via the cut umbilical vessels is a satisfactory method of obtaining blood from the neonate as it involves the minimum of disturbance and samples may be taken easily.
2. Marked hypoxia (arterial $PO_2 \simeq 19$ mm Hg) and hypercapnea (arterial $PCO_2 \simeq 55$ mm Hg) due to umbilical cord occlusion at birth become less severe (arterial $PO_2 > 45$ mm Hg, $PCO_2 \simeq 30$ mm Hg) when rhythmical breathing is established.
3. Marked changes in plasma composition occur in lambs during the first day.
4. The wide range of plasma glucose and lactate concentrations observed in animals before sucking probably reflects marked differences between the states of individual animals after birth. It was not possible to differentiate effects of birth trauma, the environment and individual responses to stress.
5. Glucose and glucogenic nutrients rapidly enter the fetal

circulation after sucking.

6. Plasma volume appears to increase after sucking possibly as a result of fluid absorption from the gastro-intestinal tract, which in one case appeared to be sufficient to compensate for blood losses resulting from severe haemorrhage.
7. Plasma corticosteroid concentrations in lambs normally decrease rapidly during the first 60 minutes after birth.
8. Plasma corticosteroid concentrations increase in postnatal lambs after haemorrhage and cold exposure.
9. Physiological changes similar to those found in lambs occur in kids during the first 12 hr after birth as assessed by changes in blood or plasma compositions.
10. Fructose appears to be produced by the placenta in goats as in sheep, since it almost completely disappears from the circulation within 24 hr of birth in both species.
11. Estimates of placental fructose synthesis before birth, based on the rate of fructose loss from a unit volume of plasma after birth, amount to only 3-6% of the glucose uptake of the fetus in late pregnancy.
12. Kids appear to have a poorer homeothermic capability than lambs of equal or lower birth weight.
13. There may be an association between plasma thyroxine concentration and the ability to maintain homeothermy in the goat.
14. The newborn must ingest milk (suck) within hours of birth if energy reserves are to be replenished and homeostasis maintained.
15. In the present study interference with the neonate was kept to a minimum and no attempt was made to regulate environmental conditions, feeding, sleeping or contact with the mother.

Further work might include controlled experiments to allow the effects of feeding, separation and environmental conditions to be more accurately assessed in the neonate.

CHAPTER NINE

THE CHRONIC APPROACH TO THE STUDY OF FETAL PHYSIOLOGY IN RUMINANTS

In the following discussion an appraisal of methods involved in studying fetal physiology in the conscious ruminant will be made, drawing on the findings and experiences of the author and the techniques and procedures adopted by other experimenters. It is not intended that this should be a criticism of others' techniques, rather a way of highlighting factors and problems which need to be considered in striving to obtain the best preparations. The problems and limitations of the 'chronic' approach are becoming increasingly apparent and several workers have emphasised these (e.g. Nathanielsz et al., 1972; Mellor and Slater, 1973a; Shelley, 1973; Comline and Silver, 1974).

A major problem in studying physiology is that the experimental study itself may change the animal and in pregnancy the fetus as well. The difficulty comes in judging whether observations made on the animal after 'interference' are representative of those in the animal before study. In conducting animal experiments this should always be borne in mind. Although in theory the effect of experimental techniques on an animal can never be eliminated the degree of disturbance can be minimised so that in practice the animal being studied, as far as can be subjectively assessed, is representative of the undisturbed animal. This is particularly important when dealing with the chronically catheterised animal which is subjected to a considerable number of procedures before and during the period when observations are made.

Two questions that immediately arise are: how are animals disturbed and how can the degree of disturbance be determined? In the context of the present work (Chapter Three), attempts were made to quantify this stress by a largely subjective assessment of changes in behaviour pattern. Stress has been associated with an increase in the secretion of hormones from the adrenal glands. In an attempt to quantify stress, changes in plasma corticosteroid and glucose concentrations and heart rate (the last two increase in association with catecholamine secretion: Setchell and McClymont, 1955; Bassett, 1970) were measured. Other workers have also measured these parameters in order to assess the effects on ruminants of environmental stresses such as climate, transport, exercise, fasting and blood sampling (e.g. Reid, 1962; Reid and Mills, 1962; Bassett and Hinks, 1969; Panaretto and Vickery, 1971; Purchas, 1973).

Tame animals can be handled more easily during experimental procedures and appear to recover more rapidly from the effects of operation (Mellor and Slater, 1973a) than untame animals. Studies have established that changes in climate, environment, diet and frequency of feeding cause marked physiological changes in ruminants (e.g. Chapter Three; Hays and Webster, 1971; Slee, 1972; Purchas, 1973; Bassett, 1974a; Bassett and Madill, 1974a). Despite this, many experimenters working with chronically catheterised animals overlook or choose to ignore the possible influences of experimental procedures including movement of their animals from one environment to another for the purposes of experiment. Experimenters may have thought the effects on their study would be insignificant and not alter their results. This is questionable since the conscious animal

is in a state of constant flux and cannot be divided into individual compartments as all changes within the animal are related.

The majority of workers using chronic techniques in ruminants do not report their pre-experimental procedures fully (e.g. Bassett et al., 1970; Dixon et al., 1970; James et al., 1972; Adamson et al., 1973; Alexander et al., 1973b; Char and Creasy, 1976) and this can make interpretation of results difficult.

Several studies have involved animals brought directly into a laboratory in which almost immediate catheterisation or sampling have been carried out (e.g. James et al., 1972; Thorburn et al., 1972). Other workers, fully aware of possible effects, have assumed that their animals had adapted to the new surroundings after a certain length of time, usually on the basis of changes in behaviour (e.g. Comline and Silver, 1970; Buddingh et al., 1971; Mellor and Slater, 1973a; Caton et al., 1974; Jack et al., 1975). The present study (Chapter Three) is one of the first attempts, by simultaneously examining behaviour and some physiological parameters, to assess the time required for animals to adapt to their surroundings. No pretence is made that the study was complete. A major limitation was that circumstances prevented all the physiological measurements being made in each group of animals and, therefore, comparison is difficult, more so because of the small number of animals involved. However, it was apparent (Chapter Three) that whatever the degree of tameness and previous experience of laboratory conditions, ruminants do require time to adjust to their surroundings and experimental procedures. This fact should be allowed for in planning an experiment

and is a principle applicable to all experiments involving conscious animals not just to chronic catheterisation.

Species differences in the time required to adapt are expected, but breed differences, which were not considered in the present study, may be marked. It is the author's experience that hill and lowland sheep have different temperaments and that even hill breeds of sheep (e.g. Scottish Blackface and Cheviot ewes) react differently to changes in their conditions. Although it is often difficult to be selective and experimenters frequently have to take what is available, unless a certain breed is specifically required, some thought should be given to the most appropriate breed when planning chronic catheterisation experiments. This should take into account temperament, source and previous handling particularly when the time available for animals to adapt to their conditions is limited by facilities or finance.

At this point it is worth emphasising that a wide variety of conditions may be expected in different laboratories in different countries where housing, weather conditions and feed as well as breeds are likely to differ. This may account partly for variations observed in sheep from different laboratories. The effects of housing, such as pen size, design, position, proximity to other animals and number of animals per pen, on the time required for animals to adapt before chronic studies begin is worthy of consideration and detailed experimental examination.

In his early studies Meschia (Meschia et al., 1965) penned his

animals individually. Most workers have followed his example (e.g. Mellor and Slater, 1971; Liggins et al., 1972; Rankin, Gresham, Battaglia, Makowski and Meschia, 1972; Thorburn et al., 1972; Currie, 1974), although catheterised animals have apparently been kept successfully in groups (Faber and Green, 1972; Strott et al., 1974). The author's experience is that group penning should be avoided, particularly if goats or horned sheep are used, otherwise catheters or other experimental equipment attached to animals may be damaged. The position of individual pens can be important as ruminants are less disturbed when they can see, hear and touch others preferably of the same species. However, the close proximity of animals, particularly goats, because of their nibbling habits, needs careful consideration if catheters are to remain intact.

Slats or mesh floors in pens, which can be kept relatively clean are an asset in reducing the risks of infection when (as in work described in Chapters Five, Six and Seven) daily sampling from fluid sac catheters is required. The fluid sacs of three of 14 goats housed on deep litter became infected with bacteria, despite using recognised and tested sampling techniques. In comparison, infection was not encountered in 12 goats subsequently housed on slats (Chapter Five). Other workers have maintained catheterised animals on bedding and have not reported problems of infection (Alexander, Britton, Mashiter, Nixon and Smith, 1970c; Rankin et al., 1972).

Most catheterised ruminants kept indoors are given a compound feed, which is a complete ration and can be easily handled and weighed. Hay is often given in addition (e.g. Comline and Silver,

1970; Shelley, 1973; Thomas, Jack, Manns and Nathanielsz, 1975) to provide the roughage content in the diet. In addition, hay given after the daily ration of pelleted feed had been consumed appeared to keep goats (Chapter Five) occupied and apparently reduced the chances of catheters being chewed.

The nutritional status of catheterised animals should be monitored because of possible effects of nutrition on the results. For example, it has been shown that variations in feed intake of a pregnant ewe has a marked effect on the glucose and fructose concentrations of fetal plasma (Shelley, 1973; Bassett and Madill, 1974a) and fetal fluids and urine (Mellor and Slater, 1973c). Few publications state the methods adopted to assess the nutritional status of the catheterised ruminant. Many workers presumably rely on a subjective assessment of body condition, or feed ad libitum (e.g. Bassett et al., 1970; Dawes et al., 1972). Measurement of weekly body weight changes and daily postabsorptive plasma glucose concentrations were used in the present study to assess nutritional status of each animal (Mellor and Slater, 1972a; Mellor et al., 1975a). Plasma FFA and ketone concentrations may also be used to assess the nutritional status of ruminants (Russell, Doney and Reid, 1967; Sykes and Field, 1972).

In this work an attempt was made to maintain animals at a level of nutrition similar to that found in the field under reasonable conditions with plasma glucose concentrations of about 500-650 mg/l. This was prefered to feeding the animals at an optimal nutritional level. Optimal levels are commonly adopted in other laboratories

although few details are given in the literature of the level of nutrition at which catheterised ruminants are maintained.

In this and other studies (e.g. Bassett and Madill, 1974b) one feed/day of concentrates was given to catheterised animals. This eliminates the extra labour involved in feeding twice daily as for example in the studies of Comline and Silver (1970) and Thomas et al. (1975). It also allows plasma or fluid samples to be taken before feeding (e.g. Chapters Five, Six and Seven) when the animals are in a relatively stable or repeatable nutritional state, as indicated by the small day to day variations in maternal plasma glucose concentrations (usually < 30 mg/l). Marked physiological changes occur at feeding. For example, Bassett (1974a) observed marked changes in plasma insulin and GH levels associated with changes in metabolites during the first six hours after feeding. Christopherson and Webster (1972) observed increases in oxygen consumption, heart rate and blood PCO_2 and decreases in blood pH and plasma volume during eating. To avoid the effects of feeding or diurnal variation the time of sampling relative to the time of feeding and time of day should be considered when designing an experiment. Problems are encountered when the duration of sampling is such that feeding interferes with or overlaps with the time of sampling. Starvation for the duration of sampling has been used in an attempt to overcome this problem (e.g. Steel and Leng, 1973). However, an alternative method would be to feed continuously, so that the animal is offered its daily ration in small feeds at set intervals throughout the 24 hr. The advantage of this method is that the marked changes in plasma parameters that are associated with once daily feeding (e.g. Blair-West

and Brook, 1969; Bassett, 1974ab) are reduced considerably (e.g. Hays and Webster, 1971; Mellor and Slater, 1973c; Hodgson and Mellor, 1977). This way of feeding has been used in metabolic studies of pregnant animals in order to keep concentrations and rates of production and utilisation of substances as stable as possible during experiments (e.g. White, Steel, Leng and Luick, 1969). It can be argued that continuous feeding will more closely approximate to the situation of the ruminant in the field than once or twice daily feeding. Clearly the merits of continuous feeding of catheterised animals and the long-term effects on fetal physiology should be examined in more detail.

Starvation for 24 or 48 hr, or a marked restriction of feed intake, is a commonly adopted procedure before ruminant surgery and, since surgery is followed by a postoperative reduction in feed intake until about the fifth to seventh day in goats and the third to fourth day in sheep (Chapter Four), good body condition at the onset of preoperative starvation is essential particularly if operations take place in the last month of pregnancy when the fetus is growing rapidly.

In general, therefore, the catheterised pregnant ruminant is housed away from adverse weather conditions, usually in an individual pen, with access to adequate feed. It has a minimal energy requirement for exercise and usually becomes accustomed to handling. It is important to remember these facts when results obtained in the laboratory are extrapolated to the field situation. Despite these limitations, laboratory control does have considerable

advantages in that the factors likely to affect animals in the field can be observed, controlled or varied individually.

After adaptation to the laboratory the next step to be considered in preparing the chronically catheterised ruminant is the operation and the effects it can have on results obtained.

In early acute experiments the stressful nature of the techniques was either overlooked or the technique itself was believed to have little effect upon the fetus, and studies were described (see Chapter One) which, when judged by today's standards appear crude. That anaesthesia and surgical trauma were associated with marked and continuous changes in maternal plasma corticosteroid concentrations (Chapter Four), confirms the suggestions (e.g. Meschia et al., 1965) that results from acute experiments should be interpreted with care. It may be argued that once surgery is completed, provided anaesthesia is properly controlled, the acute preparation can be relatively stable. For example, Comline and Silver (1974) suggest that provided maternal conditions are carefully controlled and the fetus disturbed as little as possible, data (such as pH and blood gas levels: Comline and Silver, 1970) obtained from acute experiments are often entirely comparable with that from chronic preparations. However, when a ruminant is maintained under anaesthesia for several hours it is probable that results at the beginning of the experiment will be affected by the response to surgical trauma and tissue damage (as observed in adult goats and sheep - Chapter Four) and those at the end will be influenced by changes in circulation and respiration due to accumulation of gases in the rumen and prolonged

dorsal or lateral recumbency.

As pointed out by Comline and Silver (1974) 'much pioneer work was based on these acute techniques and although experimental conditions were often far from ideal, the findings and ideas formed the basis of many subsequent investigations'. But, in future, when acute procedures are planned in a research programme they should be carefully considered and assessed to see if they are really necessary and if so, whether the results obtained from them can be interpreted and are meaningful.

The variability found in results obtained from slaughtered animals is well illustrated by the gestational changes in the composition of the fetal fluids from recently killed cattle. Thomsen and Edelfors (1976) found that the Na concentration in allantoic fluid increased during pregnancy whereas Klenov (1973) observed a decrease. In comparison, Reeves et al., (1972) found the Na concentration in allantoic fluid obtained from anaesthetised cows did not change significantly during gestation. In each of these studies variation between samples was generally large. This emphasises how misleading such measurements can be in determining for example gestational changes in fluid or plasma composition.

In early chronic studies Meschia et al. (1965) used a spinal anaesthetic during surgery. However, since then a wide variety of anaesthetics have been used. In the present work pentobarbitone sodium was used both to induce and maintain anaesthesia. This was chosen because it was in general use in the laboratory and had no

apparent effects on the viability of the ovine fetus after operation (e.g. Comline and Silver, 1970; Mellor and Slater, 1971, 1972). Fletcher, Rogers and Donaldson (1964) found individual differences in the responses of goats to pentobarbitone sodium and a narrow margin between anaesthetic and lethal doses, but suggested that the anaesthetic was satisfactory provided it was carefully administered. Apnoea, as found in goats anaesthetised with pentobarbitone sodium by van Rensburg (1971), did occur in some goats in the present study after induction of anaesthesia. These goats were then ventilated manually until the end of the operation when breathing was re-established. Apnoea and postoperative abortion were not associated. Other techniques commonly used to maintain anaesthesia during surgical insertion of uterine catheters have involved the use of epidural (e.g. Liggins et al., 1973) and inhalational anaesthesia (e.g. halothane: Dixon et al., 1970; Thorburn et al., 1972; Drost et al., 1973; Currie, 1974; Flint et al., 1974).

When considering the effects of operation on goats and sheep (Chapter Four) no attempt was made to differentiate between effects of various anaesthetics on the pregnant ruminant and its fetus although differences have been reported (Copland, 1976; Green and Moor, 1977). This was a limitation and it is worth consideration, particularly in view of the incidence of postoperative abortion in the present goats. Comline and Silver (1970) found that severe fetal acidosis was associated with closed-circuit halothane anaesthesia in their first series of experiments. Umbilical catheters remained patent for three days or more in only 12 of 34 sheep and a high proportion of the failures aborted soon after operation. In subsequent

operations (Comline and Silver, 1970) on 17 sheep pentobarbitone sodium was used, fetal acidosis was less marked and umbilical catheters remained patent for more than three days in 10 animals. Halothane has been extensively used during surgical catheterisation (see above) with no apparent detriment to the fetus. The findings of Comline and Silver (1970) may indicate that halothane is a hazardous anaesthetic for sheep or that their surgical technique had improved, or both. In the present work, when applying a new catheterisation method to the fetus (Chapter Six) improvements in surgical technique, which reduced the length of the operation and the amount of manipulation of the fetus, occurred as experience was gained. These factors would be expected to affect the viability of the fetus and the number of animals successfully maintained for long periods. This occurred in the present study despite a thorough trial of the technique using slaughtered material.

The importance of asepsis during the operation and subsequent sampling period rather than direct antibiotic treatment of mother and/or fetus at operation has been emphasised by others (e.g. Nathanielsz et al., 1972; Mellor and Slater, 1973a; Comline and Silver, 1974). Routine antibiotic treatment is usually given to most animals for up to five days after surgery and this practice was adopted in the present work. In addition, exogenous progesterone treatment of sheep and goats before operation is an accepted method of reducing myometrial contractions after the uterus has been handled. Currie (1974) found that exogenous progesterone treatment around the time of surgery gave 'sufficient protection from surgically induced abortion'. In contrast, in the present study

exogenous progesterone treatment of goats at operation did not reduce the incidence of postoperative abortion. If abortion is caused by an increase in utero-ovarian $PGF_2\alpha$ (see Chapter Five), treatment of goats with inhibitors of prostaglandin synthesis (e.g. indomethacin, acetyl salicylic acid or flufenamic acid) around the time of operation may be a good alternative to progesterone in the prevention of postoperative abortion.

The surgical techniques adopted by experimenters will depend to a large extent upon personal preference and experience. No attempt is made here to discuss the merits or limitations of general surgical techniques and approach to the fetus. However, attention must be drawn to a number of factors concerning techniques which relate to the normality of the chronic preparation.

After surgery, the main problem comes in attempting to determine when a preparation can be regarded as representative of the 'normal' conditions of gestation. Comline and Silver (1974) point out that 'often the need for recovery is ignored and insufficient reasons are given for the assessment of a normal fetus'. Wide variations in the recovery times allowed by other workers (see Chapter Four) may reflect differences in operative procedures and gestational age at operation but also certainly reflects differences in the criteria used to judge recovery. In the present study (Chapter Four) it became apparent that maternal plasma glucose concentrations did not reach values in the pre-operative range until after 10-12 days in goats and 5-7 days in sheep. Marked fluctuations in plasma amino acid concentrations occur for 6-12 days after operation in pregnant

sheep (Slater and Mellor, 1977). Other parameters (plasma osmolality and K, urea and corticosteroid concentrations) stabilised within 2-5 days of operation or did not change (Na and Cl concentrations) after operation (Chapter Four; Bassett and Thorburn, 1969; Mellor and Slater, 1971; Hoffmann, Wagner and Giménez, 1976). Changes in fetal fluid and urine composition were also observed for up to 14 days after operation in goats and sheep (Chapter Five; Mellor and Slater, 1971, 1972a). Gresham et al. (1972) reported that rates of fetal urine secretion did not stabilize until 3-6 days, while Shelley (1973) found in her best preparations that fetal plasma concentrations of glucose, fructose and lactate stabilized in 3-5 days. Dawes et al. (1972) reported that fetal breathing stabilized after about two days.

Despite these findings, while different recovery times are allowed in different laboratories, several workers using catheterised animals fail to report the time after operation when measurements on 'recovered' animals begin (Rankin et al., 1972; Drost et al., 1973) and others report recovery after less than 1-2 days (Willes, Boda and Manns, 1969; Dixon et al., 1970; Tsoulos et al., 1971). While some changes have stabilized after this period the animal cannot have recovered completely in this time. Experiments conducted within a few days of operation should be viewed with the same caution as acute experiments. It is difficult to generalise, as conditions in laboratories vary, but a minimum of seven days should be allowed for sheep and 12 days for goats, so that incisions have had time to heal, the animals can move around without apparent discomfort and return to preoperative voluntary feed intakes. When contemplating metabolic studies of the fetus it is worth considering a longer recovery

period (16 days are required for allantoic fluid and fetal urine hexose concentrations to stabilise after operation: Mellor and Slater, 1972a, 1973b) if results are to be related to the conscious, unoperated, unstressed animal. Shelley (1973) and Slater and Mellor (1977) emphasise the dangers of conducting metabolic studies in sheep before they have recovered from effects of operative procedures.

Unfortunately, in early pregnancy (before about 60 days of gestation in sheep and goats) placental blood vessels are fairly small and fetal tissues are too fragile to allow direct fetal surgery with good fetal survival. Although the fetal fluid sacs of the sheep have been successfully catheterised at 60 days (e.g. Mellor and Slater, 1971) with the preparation going to term, surgical insertion of catheters into the conceptus is usually limited to the later half of gestation that is from about day 80 in sheep (e.g. Comline and Silver, 1970). This is after most of the placental growth is complete but before the rapid increase in fetal growth takes place at the end of pregnancy (Cloete, 1939; Barcroft, 1946; van Rensburg, 1971).

The normality of preparations operated on in the last two weeks of pregnancy (e.g. Anderson and Turnbull, 1972; Wilson et al., 1973) is suspect as parturition can occur prematurely and it is difficult to visualise how postoperative recovery could be completed before changes associated with parturition occur. This is important, particularly if, as is usually the case, these preparations are being used to examine the physiological changes associated with

parturition. Several examples are available in the literature of studies on parturition induced prematurely within a week of surgery; Flint et al., (1974) studied changes of dexamethasone induced parturition at most 67 hr after surgery in late pregnancy. The gestational age at which catheters are to be inserted into animals in which prepartum changes are to be examined is often influenced by the period catheters, usually in the fetal vasculature, will remain patent. If such technical difficulties do not allow adequate time for postoperative recovery the particular technique used to obtain fetal blood should be reconsidered and probably changed. In cases where the only technique available is an unreliable one, a compromise must be struck between the need for adequate postoperative recovery and the patent life of the catheter. For example Currie (1974) found it necessary to prepare goats at earlier stages in pregnancy than sheep in a similar study in an attempt to minimise surgical trauma of the uterus. However, the likelihood of his utero-ovarian vein catheters remaining patent was proportionately reduced.

Surgical insertion of catheters for pregnancy studies in goats and sheep and to a lesser extent cattle is limited to a relatively short period in the reproductive cycle. This means that if artificial methods of lengthening or changing the breeding season are not adopted, careful planning is necessary to ensure that the gestational ages of the animals are sufficiently spaced to allow each to be catheterised at the most favourable time.

Because of the effort and expense involved in maintaining catheterised animals and, particularly if housing facilities are

limited, it is understandable that some experimenters subject the fetus to extensive surgery and insert a number of catheters into the conceptus during an operation which must last several hours. For example Boddy, Dawes and Robinson (1973) implanted tracheal and carotid catheters, bi-parietal cortical electrodes and a tracheal electromagnetic flowmeter into 23 fetal lambs between 97 and 137 days in gestation and recorded results from 57 hr after operation. Other preparations involving several catheters in the conceptus have also been reported (e.g. Gresham et al., 1972; Bradley and Mistretta, 1973; Shelley, 1973; Hoffmann et al., 1976; Thornburg, Bissonnette and Faber, 1976). Whether, after operation, these preparations ever recover sufficiently to enable results to be obtained that are comparable with those that would be obtained in the unstressed, unoperated animal is questionable. Where possible every attempt should be made to limit the number of catheters and degree of surgical trauma imposed directly on the fetus.

On a similar note, the validity of results obtained from animals re-operated on after catheters have pulled out or blocked (e.g. Alexander et al., 1973b) must be seriously doubted. It is surely better to have alternative experiments planned for such contingencies.

Catheterisation procedures may have adverse effects on the fetus. This is true of some techniques used for vascular catheterisation. Catheterisation of blood vessels in the hindleg of the fetus can cause stunting or abnormal limb development

(Alexander et al., 1973b; Mellor and Matheson, 1975) and abnormalities of brain development can occur after carotid artery ligation in fetal sheep (G.D.Thorburn, personal communication).

Once the ruminant has recovered, sampling becomes the critical procedure. As indicated in Chapter Three if animals are moved from holding pens to a laboratory for sampling (e.g. Strott et al., 1974; Char and Creasy, 1976) this should be included in any adaptation schedule. Various methods have been used to protect and to sample from catheters and all are designed to prevent infection of the conceptus via the catheters. Mellor and Slater (1973a) point out that the introduction of large quantities of heparin into the fetal circulation (e.g. Meschia et al., 1965) results in disturbances of membrane function and haemorrhage into the fluid sacs. Recently reported sampling techniques introduce smaller amounts of heparin into the fetus (e.g. Comline and Silver, 1972; Mellor and Matheson, 1975). Sampling techniques that are suited to one laboratory may not be appropriate in another where differences in environment and housing conditions exist.

It is worth considering whether fluid or blood samples are representative of the fluid space from which they were obtained. The composition of allantoic fluid samples from each end of the allantoic sac were similar in a goat (Chapter Five) and in sheep (Mellor and Slater, 1971) but fluid obtained from amniotic sac catheters may not be representative of the sac as a whole because the fetus, particularly in late pregnancy, is likely to prevent complete mixing of fluid. Changes observed in amniotic fluid in the

last 1-2 weeks of pregnancy in goats (Chapter Five) and sheep (Mellor and Slater, 1971) might reflect the increasingly mucoid nature of the fluid since only the watery fraction of the fluid could be analysed. Catheters well placed in the fetal vasculature sample a continuously moving stream of fluid, but differences in blood composition in different vessels (e.g. Dawes, 1968) must be considered.

Frequency of sampling has to be a compromise between availability and the changes under observation. Once-daily sampling should be more than sufficient to observe gestational changes in fetal fluid or plasma composition provided conditions at sampling are kept as consistent as possible (e.g. Bassett et al., 1970; Mellor and Slater, 1971; Alexander et al., 1973b). But when particularly rapid changes are occurring as for example after feeding or before and after parturition, more frequent sampling is necessary (e.g. Edwards, 1970; Arije et al., 1974; Bassett and Madill, 1974ab; Comline et al., 1974). Whatever the frequency of sampling decided upon, repeated sampling from patent vascular catheters in the fetus is severely limited by the small blood volume of the fetus. Removal of large volumes of fetal blood is sometimes associated with premature delivery or fetal death (Mellor and Matheson, 1975; Rees et al., 1975). Sampling from the fluid sacs, because of a relatively rapid turnover of fluid, is less of a problem. In the adult, blood volume imposes less of a limit to sampling frequency and catheter potency is not a problem when sampling blood from the peripheral circulation. This has enabled techniques of continuous blood sampling to be developed which allow the short-term but marked changes in plasma concentrations of

pituitary hormones to be observed in ruminants (W.R.Carr, personal communication). Daily samples can only give a restricted view of short-term changes occurring in for example plasma LH concentrations (Chapter Five; Arije et al., 1974) or ACTH concentration (Rees et al., 1975).

Infusion or injection of substances into the conceptus enables their effects in the fetus to be examined. Every effort should be made to ensure that the amounts of substances given are in the physiological range although initially it may be necessary to infuse a greater amount so that an effect can be detected. Asepsis is important if results from infusions are to be determined with certainty. For example, infusion of glucose at 70 mg/min into fetal lambs in the last few weeks of pregnancy was associated with fetal death within 24 hr in some sheep (Bassett and Madill, 1974b). This could have been an effect of the rate of infusion or infection of the fetus. Obviously, as stated above, animals should have recovered from operation before substances are introduced. The introduction of substances into the conceptus at operation (e.g. Flint et al., 1974) or within 1-2 days (Setchell et al., 1972; Liggins et al., 1972) is to be avoided.

If every attempt is made to reduce the effects on the animal of the procedures discussed above then the chances of gross abnormalities arising in the results from the chronic procedures themselves will be considerably lessened.

A comparison of catheterised and uncatheterised animals during

gestation to assess the condition of the former (e.g. Chapter Five; Comline et al., 1974) is limited to behaviour and plasma composition of the mother and gives little indication of conditions in utero. In animals with fetal vascular catheters stability of blood gas, pH and PCV values and fructose, lactate and glucose concentrations in fetal plasma have been used as indices of fetal wellbeing (viz. Nathanielsz et al., 1972; Shelley, 1973; Comline et al., 1974; Comline and Silver, 1976). Bradley and Mistretta (1973) noted that fetal swallowing stopped three days before death in utero and Mellor and Slater (1974) note that a decrease in fetal urine pH also precedes fetal death. Plasma corticosteroid concentrations have been used as an index of stress in the mother (Chapters Three and Four) but this may not be applicable in the fetus as plasma corticosteroid concentrations in the ovine fetus remain low, as does the adrenal response to ACTH, until late gestation (see Chapters Seven and Eight). It is worth noting that Alexander et al., (1973b) observed circulating concentrations of ACTH above 200 pg/ml in the plasma of some chronically catheterised fetuses but not in acutely exteriorised fetuses and suggested that the fetuses in these chronic preparations may be stressed. Rees et al., (1975) found ACTH concentrations of 180-700 pg/ml in daily fetal plasma samples while Jones (1975) reported fetal values of 50-200 pg/ml. The reasons for these differences remain to be clarified.

In cattle and goats, application of chronic techniques have been less successful than in sheep. It appears that goats are more readily disturbed by the catheterisation procedures and take longer to recover from their effects than sheep. The high incidence of

postoperative abortion in goats (Chapter Five) emphasises the difficulty of applying techniques successful in one species to another. In cattle, catheterisation of the conceptus is often associated with premature parturition and retained placenta. Differences are also apparent in prepartum hormonal changes compared to those in uncatheterised cattle (Comline et al., 1973; Comline et al., 1974; Comline and Silver, 1976).

In the present work few animals (Chapters Five, Six and Seven) had vascular catheters, making it difficult to assess fetal well-being. So assessment of a 'normal' catheterised preparation was facilitated by the final test of parturition, which as stated by Nathanielsz et al., (1972) should be 'the desired goal of all chronic catheter work'. Gestational age at birth, birth weight and the development and viability of the newborn were carefully monitored. As a healthy lamb has a continuous weight gain which begins on the first day after birth (Nathanielsz, 1970), performance to weaning was also assessed. These factors can vary considerably in unoperated animals and the author is aware of the limitations in making the assumption that viability at birth reflects normality in utero. Other experimenters report the condition of catheterised animals at birth (e.g. Alexander et al., 1974b; Comline et al., 1974; Currie, 1974; Mellor and Matheson, 1975). However, such postpartum observations have only rarely included changes in blood composition. The technique outlined (Chapter Eight) eliminates the necessity for pre- or post-partum surgery, and the trauma of repeated venepuncture in the newborn. Unfortunately this technique could not be applied until late in the course of the present work so postpartum changes

in blood composition were not measured in all of the animals from which intrauterine data were obtained.

Finally to summarise the following are the important factors to be considered in studying conscious ruminants particularly if the degree of disturbance caused by the experimental procedures is to be minimised:

Animals should have adapted to their surroundings, diet and experimental procedures before experiments begin. Even tame ruminants can take seven days to become accustomed to a new diet.

If surgery is involved then recovery from the surgical procedures is also essential and in pregnancy this includes fetal recovery. It is difficult to generalise because of the different conditions in various laboratories and the different parameters studied but on the basis of the present work a minimum of seven days for sheep and 12 days for goats should be allowed for recovery from the immediate effects of operation.

When techniques allow, surgery should be carried out at a sufficiently early stage in pregnancy to allow the desired observations to be made in recovered, unstressed animals. Techniques which involve extensive fetal surgery and those which cause long term effects on fetal growth and development are to be avoided.

Effects of sampling, and infusion techniques should be evaluated critically and procedures modified when necessary.

Stability of, for example, fetal blood pH and corticosteroid, fructose and lactate concentrations can be used as an index of fetal wellbeing.

Birth of a viable neonate need not indicate normality in utero. However, gestational age at parturition, delivery of the newborn, birth weight, development and viability at birth and during the first 24 hr of life and growth rate to weaning can provide some indication of normality in utero.

In conclusion, the chronic approach is widely used and at present is the most acceptable way of studying fetal physiology. As with any method it has limitations but it is possible with care to ensure that chronic preparations are of a high standard so that results obtained relate to the conscious animal.

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APPENDIX

PUBLISHED WORK.

The following papers have been published. A reprint of each is appended:

D.J.MELLOR and R.A.PEARSON (1974). Changes in ionic composition of allantoic fluid during adrenocorticotrophin infusion into fetal sheep. Research in Veterinary Science. 16 108-109

R.A.PEARSON and D.J.MELLOR (1975). Some physiological changes in pregnant sheep and goats before, during and after surgical insertion of uterine catheters. Research in Veterinary Science. 19 102-104.

R.A.PEARSON and D.J.MELLOR (1976). Some behavioural and physiological changes in pregnant goats and sheep during adaptation to laboratory conditions. Research in Veterinary Science. 20 215-217

R.A.PEARSON and D.J.MELLOR (1976). The composition of ruminal and abomasal fluid from catheterised fetal sheep during the last 50 days of pregnancy. Research in Veterinary Science. 21 100-101

R.A.PEARSON and D.J.MELLOR (1977). Changes in fetal fluid composition during the last 60 days of gestation in goats. Journal of Reproduction and Fertility. 50 (in press)

Res. vet. Sci. 1974, **16**, 108–109

Changes in Ionic Composition of Allantoic Fluid during Adrenocorticotrophin Infusion into Fetal Sheep

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SUMMARY. Intravenous infusion of synthetic adrenocorticotrophin (ACTH) at rates of 1·0 or 4·0 μg/h into 3 chronically catheterized fetal sheep coincided with a fall in sodium and a rise in potassium concentrations of allantoic fluid which continued until premature delivery of the lambs. This is consistent with an association between changes in activity of the fetal pituitary-ACTH-adrenal axis and variations in the ionic composition of allantoic fluid.

DURING THE LAST 70 days of pregnancy in sheep (term at about 147 days) the sodium concentrations of allantoic fluid generally decrease and simultaneously the potassium levels increase (Mellor & Slater, 1971). In normoglycaemic ewes these ionic changes start at about 120 days gestational age (Mellor & Slater, 1972) and parallel those of the corticosteroid concentrations of fetal plasma (Bassett & Thorburn, 1969; Comline *et al.*, 1970; Drost *et al.*, 1973). In hypoglycaemic ewes similar ionic changes start at earlier gestational ages (Mellor & Slater, 1971; 1972). It has been suggested, therefore, that the sodium and potassium concentrations of allantoic fluid are regulated by fetal plasma corticosteroids acting on pumping mechanisms in the chorioallantois, and that maternal-induced fetal hypoglycaemia increases fetal corticosteroid secretion. An inverse relation between the glucose and corticosteroid concentrations of fetal plasma during insulin infusion into fetal sheep (Liggins *et al.*, 1973) agrees with this, but a direct association between changes in adreno-cortical secretions and allantoic fluid composition has yet to be established. The present study was designed to test this association.

MATERIALS AND METHODS

Three 4-year-old Scottish Black Face ewes (48–57 kg) carrying 4 lambs of known conceptual age were used. The housing, husbandry, maintenance and nutritional monitoring procedures were as described by Mellor & Slater (1972). In 3 fetuses a catheter was inserted into the allantoic sac using the technique of Mellor (1970a) and into the umbilical vein draining the pregnant uterine horn *via* a small vessel from a cotyledon located on the ventral surface of the body of the uterus (Mellor, Maule Walker & Pearson, unpublished modification of the method of Meschia *et al.*, 1965). All other surgical details, and catheter maintenance and sampling techniques were as described by Mellor & Slater (1971).

Synthetic ACTH* was dissolved in sterile 5% (w/v) glucose solution which was infused into the umbilical venous catheter of each fetus at 0·67 ml/h, with ACTH concentrations allowing delivery rates of 1·0 or 4·0 μg/h. The sodium and potassium concentrations of allantoic fluid and the glucose levels of maternal plasma were determined as before (Mellor & Slater, 1972).

RESULTS AND DISCUSSION

The ACTH infusion was allowed to continue until premature delivery of the lambs, which were all alive at birth (Fig. 1). The lamb from ewe B209 and the uninfused twin from ewe B203 died within 1 h, but the other 2 survived for at least 35 days. The birth weights of 1·7 kg (B209), 2·6 kg (B211), 2·3 kg (uninfused; B203) and 2·6 kg (infused; B203) were normal for the stage of gestation, and the weights of the adrenal glands of the dead lambs were within the well established ranges for infused and uninfused fetuses delivered prematurely in this way (Liggins, 1968; Nathanielsz *et al.*, 1972). Infusion of ACTH into fetal sheep at 4–10 μg/h causes adrenal gland hypertrophy and a progressive increase in the secretion and plasma concentrations of corticosteroids, which finally results in premature delivery (Liggins, 1968; Liggins *et al.*, 1973; Bassett & Thorburn, 1973). In all 3 fetuses, ACTH infusion at 1·0 or 4·0 μg/h was associated with a fall in sodium and a rise in potassium concentrations of allantoic fluid which continued until birth (Fig. 1). Immediately before and during infusion in each animal the glucose concentrations of maternal plasma remained relatively constant (B209, 46–52 mg/100 ml; B211, 50–55 mg/100 ml; B203, 37–41 mg/100 ml) so the infusion is the likely cause of the ionic changes. Since ACTH itself is not known to act directly on ion pumps these changes probably resulted from ACTH stimulation of corticosteroid secretion. It is not suggested, however, that the marked rise in fetal plasma concentrations of corticosteroids observed during the 4–7 days before natural and ACTH-induced parturition (rising from about 3 to 15–25 μg/100 ml; Comline *et al.*, 1970; Bassett &

* Synacthen, Ciba Laboratories, Sussex.

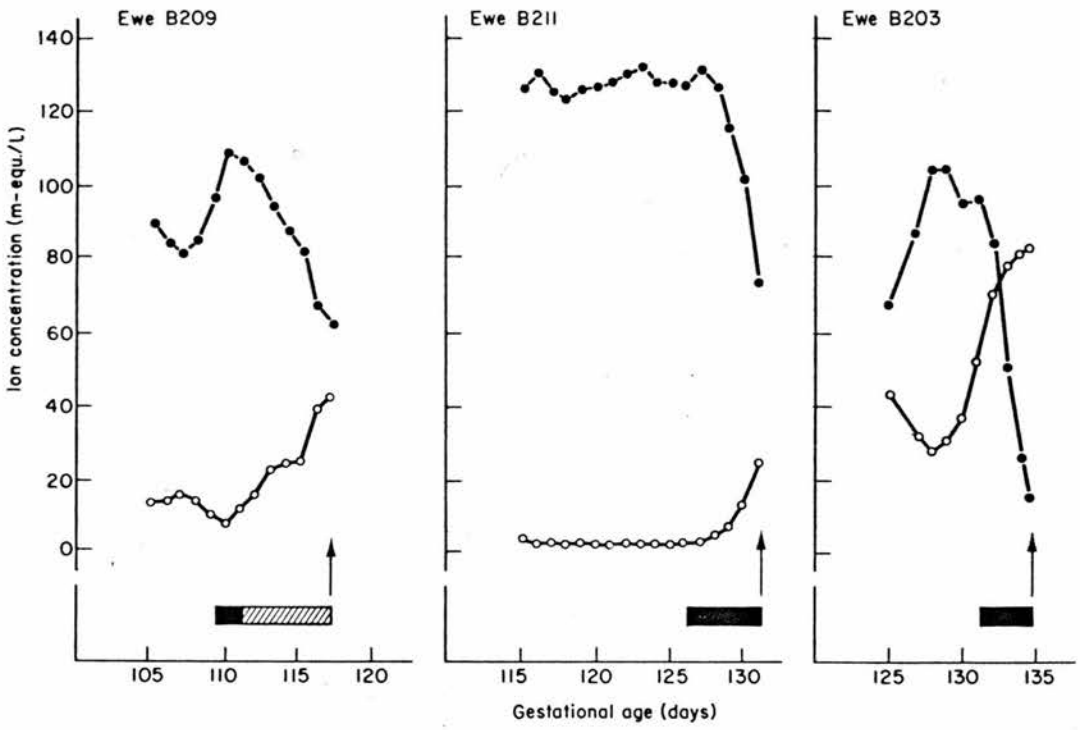

FIG. 1. Changes in sodium (●) and potassium (○) concentrations of allantoic fluid from 3 fetuses which were infused intravenously with ACTH at rates of 1·0 μg/h (solid bar) or 4·0 μg/h (striped bar) until delivery (arrow). Gestational ages at operation were 103 days (B209), 107 days (B211) and 121 days (B203).

Thorburn, 1969; 1973; Drost *et al.*, 1973) is necessary to stimulate these ionic changes in allantoic fluid. In fact, corticosteroid concentrations of fetal plasma rarely exceed 3–5 μg/100 ml until 4–7 days before birth (Bassett & Thorburn, 1969; 1973; Drost *et al.*, 1973), and maternal hypoglycaemia at any stage after 90–100 days gestational age induces changes in the ionic composition of allantoic fluid of the same or a greater magnitude than those observed here without causing premature delivery (Mellor & Slater, 1971; 1972). Therefore, the ionic pumping mechanisms in the chorioallantois (Mellor, 1970b) probably respond to relatively small variations in fetal plasma levels of corticosteroids.

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Some physiological changes in pregnant sheep and goats before, during and after surgical insertion of uterine catheters

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Corticosteroid concentrations in maternal plasma from pregnant sheep and goats increased during the pre-operative starvation period, anaesthesia and surgery, and reached maximum values when the animals regained consciousness. During the operation the vaginal temperature fell 2–3°C. Postoperatively, corticosteroid levels took two to four days, while glucose concentrations took five to seven days in sheep and 10–12 days in goats to return to prestarvation levels. Feed intakes returned to prestarvation levels three to four days after operation in sheep and five to seven days after operation in goats.

EARLY STUDIES OF fetal ruminants have usually been made on anaesthetised dams that have been surgically manipulated to allow access to the uterus. The presumed stress of these 'acute' procedures has been avoided by the use of 'chronic' catheterisation techniques that allow fetuses to be investigated in conscious dams that have recovered from uterine surgery. The surgical procedures used in both types of experiment are similar. However, while some differences between fetuses examined under acute and chronic conditions have been found (eg, Meschia *et al* 1965; Comline and Silver 1970), no direct evidence that acute procedures stress the mother is available. We have therefore followed changes in plasma corticosteroid concentrations in sheep and goats before and during surgical insertion of uterine catheters for subsequent chronic observations. In addition, since estimates of recovery time in catheterised sheep vary from 2–3 h (Dixon *et al* 1970; Setchell *et al* 1972) to three to five days (Shelley 1973) and since there are no estimates for goats, we have also followed postoperative changes in plasma corticosteroid and glucose levels and in feed intake in both species.

Materials and methods

Eight pregnant Scottish Blackface ewes (50–70 kg) and four pregnant British Toggenburg × mixed breed goats (40–60 kg) of known conceptual age were used. All animals were fed and maintained as described by Mellor and Slater (1972) except that goats were kept on deep litter and were offered hay in addition to the daily ration of pelleted feed*. The animals were handled daily, including sampling of blood from a jugular vein, for six to eight weeks before operation at 89–114 days (sheep) or 81–98 days (goats) gestational age. Maternal jugular blood was sampled before feeding each day, or at 0900 h on the two days preceding surgery, except in five animals, when sampling was more frequent on the day of operation. All animals were fasted for 48 h before operation.

Operative procedure

In four sheep and one goat blood was sampled at the end of each stage of the operation. The time of sampling measured from when the animal entered the surgery has been shown in brackets in the following description. The course of the operation was the same in all 12 animals in this study.

Stages (*Fig 1A*). Blood sampled in the animal house (A, 0 h); a jugular vein catheterised under local anaesthetic† in the surgery (B, 0·1 h); anaesthesia induced with, and kept at surgical depth by additional intermittent doses of sodium pentobarbitone‡, an endotracheal tube inserted into the trachea and the animal placed on the operating table (C, 0·3 h); animal connected to pure oxygen, its abdomen prepared and a skin incision made as described by Mellor and Slater (1971) (D, 0·9 h); laparotomy, exposure of the uterus, catheters inserted

* Ruminant A Diet. Seafield Mill, Penicuik, Midlothian.
† Lignostab. Crookes Veterinary Ltd, Basingstoke.
‡ Nembutal. Abbott Laboratories, Kent.

and uterus returned to the abdomen as previously described (Mellor *et al* 1972) (E, 1·7 h); the peritoneum, muscle layers and skin sutured (F, 2·3 h); the endotracheal tube removed and the animal placed in a warm pen (G, 3·2 h); finally, the animal conscious and attempting to stand (H, 5·3 h). At intervals during the operation the animal's temperature was measured at a depth of 18 cm within the vagina. Anaesthesia was induced with a mean of 24 mg sodium pentobarbitone/kg body weight in the sheep or 18 mg/kg in the goats and during the following 1·7–2·0 h it was maintained with a further 23 mg/kg in sheep or 21 mg/kg in goats.

Analytical procedures

Plasma corticosteroid levels were measured by the method of Bassett and Hinks (1969) modified as described by Mellor *et al* (1975) and glucose levels were determined according to Mellor and Slater (1972).

Results and discussion

Changes during operation

In four sheep and one goat (Fig 1A), plasma corticosteroid levels rose throughout the operation reaching about 100 ng/ml when final suturing was complete. Cessation of surgical stimulation resulted in a transient fall to about 70 ng/ml followed by a rise to about 120 ng/ml when the animals were conscious. Although no attempt was made to separate the effects of anaesthesia and surgery these results suggest that a major cause of the rise in corticosteroid levels was surgical trauma.

In all five animals, intravaginal temperatures decreased during the operation from 38–39°C to 35–36°C, the largest fall occurring when sterilising solutions were applied to the abdomen. Temperatures only returned to preoperative values 6·0–6·5 h after the start of operation. In six other sheep, similar temperature reductions occurred during acute experiments which continued for 3 h or more (D. J. Mellor, unpublished data). This hypothermia together with the high plasma corticosteroid levels in anaesthetised, surgically manipulated animals (Fig 1) complicates interpretation of results from acute experiments since most of them have been performed under similar conditions (Dawes 1968).

Changes before and after operation

In another four sheep and in four goats (Fig 1B) plasma corticosteroid levels increased and glucose values decreased during the 48 h preoperative fast. This confirms observations in sheep (Purchas 1973; Bassett and Madill 1974). In both species feed intakes were usually between

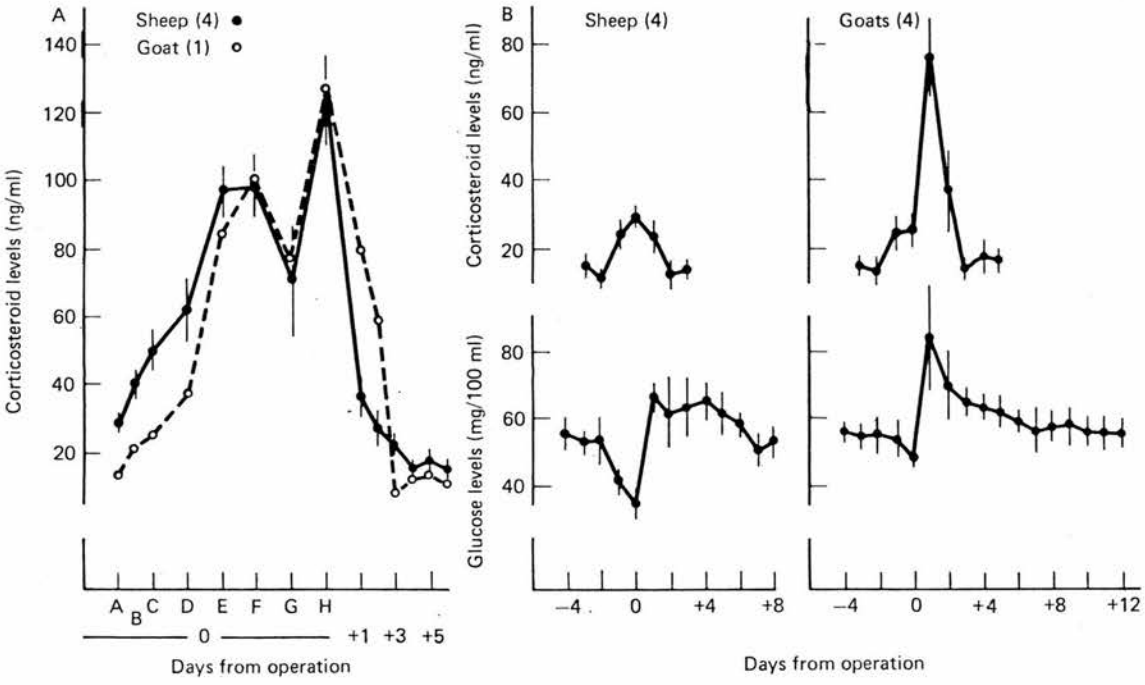

FIG 1: A. Changes in plasma corticosteroid levels during surgery (see text for description of stages A—H) and the following six days in four sheep (mean ± SE) and one goat. B. Daily changes in mean (± SE) plasma corticosteroid and glucose concentrations in four sheep and four goats around surgery

10 and 50 per cent of prefasting values until the second day after surgery. In sheep, intakes returned to prefasting values (ie, 0·9 or 1·4 kg pelleted feed/day) on the third or fourth day after surgery, while in goats the intakes reached prefasting levels (ie, 1·0 kg pelleted feed plus 0·3–0·5 kg hay/day) five to seven days after operation.

In both groups of sheep, mean plasma glucose concentrations stayed higher than prefasting values until seven days after operation (Fig 1B). However, mean corticosteroid levels remained significantly higher ($P = 0·01$) than base line values (12–16 ng/ml) for only three days in one group (Fig 1A) and one day in the other group (Fig 1B). This suggests that stress during recovery was greater in the first group which was catheterised at fetal ages of 104–114 days, 15–20 days later in pregnancy than the second group. In the four goats, corticosteroid and glucose levels on the first two days after surgery were greater than those in the sheep (Fig 1B), and goat plasma glucose levels took 10–12 days to return to prefasting values. This suggests that postoperative stress was greater in the goats than in the similarly treated sheep.

Data obtained from experiments done within two to three days of surgery (Flint *et al* 1974) while corticosteroid levels are likely to be elevated (Fig 1) probably apply to stressed animals with disturbed feeding patterns. Statements that animals had 'recovered' 2–3 h after surgery (Dixon *et al* 1970; Setchell *et al* 1972) are not supported by the results of the present work. Indeed, recovery of the mother seems to require at least seven days in sheep or 12 days in goats, and other work in this laboratory has shown that sheep fetuses require up to 16 days for electrolyte and hexose stability to be reached postoperatively (Mellor and Slater 1972, 1973).

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Some behavioural and physiological changes in pregnant goats and sheep during adaptation to laboratory conditions

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Eight goats and 12 sheep were examined during their first six to eight weeks in the laboratory. Plasma corticosteroid concentrations in four untamed goats and four untamed sheep were elevated during the first week but thereafter remained at basal values. In contrast, low corticosteroid concentrations were observed throughout in four tame goats and in four untamed sheep sampled only after they had been in the laboratory for two weeks. Plasma glucose concentrations were elevated during the first week in the untamed goats.

In four partially tamed sheep a rise in heart rate which occurred transiently during handling for 4–6 min did not occur when these animals showed tame behaviour after two to three weeks, and in four untamed sheep a rise in plasma corticosteroid concentration, which occurred transiently when seven blood samples were taken in 90 min, did not occur when these sheep were judged to be tame after five to six weeks.

IN THIS LABORATORY goats and sheep are generally brought into experimental rooms directly from the field. At first most animals are highly excitable and they require time to adapt to their new surroundings. The process of adaptation may be regarded as having two components; (1) adaptation to experimental rooms, the new diet and management procedures, and (2) adaptation to being handled directly during repeated blood sampling or other prolonged experimental procedures. We have studied the behaviour, and measured feed intake, plasma glucose and corticosteroid concentrations and heart rate of pregnant goats and sheep during their first six to eight weeks in the laboratory and have attempted to differentiate between effects of the two components of the adaptation process.

Materials and methods

Animals

Eight two- to seven-year-old British Toggenburg cross mixed breed goats (40–70 kg) and 12 four- to six-year-old Scottish Blackface ewes (50–70 kg) were brought into individual pens in the laboratory 30–40 days after mating. Four ewes each had a carotid loop. All animals were fed 0·8–1·0 kg pelleted feed*/day and in addition the goats received 0·3–0·4 kg hay/day.

Degree of tameness

Four of the goats, designated 'tame', were bottle reared and were accustomed to being handled, while the other four 'untamed' goats were used to seeing attendants but not to being handled. The four sheep with carotid loops had become used to handling during a three month period which ended six months before the start of the experiment, and were therefore described as 'partially tamed'. The other eight 'untamed' sheep were brought in directly from the field and were not accustomed to being handled.

Handling and criteria of tameness

All animals were handled daily for 5–10 min. This consisted of stroking their necks and heads and quietly talking to them. Untamed behaviour was characterised by the animals remaining alert at the back of the pen or rapidly circling or attempting to jump out of the pen when approached. When handled directly untamed animals displayed extreme muscular tension and responded to stroking by attempting to escape, or by butting and stamping. When tame these characteristics were completely absent; when approached for blood sampling the animals would come to the front of the pen and stand quietly, often cudding, while being held for sampling.

Measurements

In the four ewes with carotid loops heart rate was measured for 1 min using each of the following procedures: heart rate 1 (HR_1)—counted from outside the pen by observing pulses in the carotid loop; heart rate 2 (HR_2)—counted immediately after entering the pen while gently holding the carotid loop; and heart rate 3 (HR_3)—counted after a further 3–5 min of talking to and gently stroking the ewe. Heart rates were measured at 0830–0900 h before daily laboratory activities started and before the animals were fed.

Plasma glucose concentrations were measured as described by Mellor and Slater (1972) and corticosteroid concentrations according to Mellor *et al* (1975).

Blood sampling

Maternal jugular blood was taken from all animals except the four sheep with carotid loops before feeding and usually within 10–15 s of entering each pen. In most cases single daily blood samples were taken. These were taken quickly before the glucose and corticosteroid concentrations in jugular blood could be affected by the sampling procedure. Repeated venipuncture experiments were performed at weekly intervals for five weeks. In each experiment blood was sampled at 0, 7, 15, 30, 45, 60 and 90 min.

* Ruminant A Diet. Seafield Mill, Penicuik, Midlothian

Results

Experiment 1

It took up to one week before all the goats ate the whole of their daily ration of pelleted feed. The behaviour of the four tame goats did not change, but the four untamed goats became progressively less excitable until after two to three weeks their behaviour was indistinguishable from that of the tame group. Plasma glucose and corticosteroid concentrations were elevated in the untamed group during their first five to nine days in the laboratory, but then decreased to values which were similar to those maintained by the tame group throughout the 28 day sampling period (Table 1).

TABLE 1: Changes in mean (±SE) plasma levels of glucose and corticosteroids in four untamed and four tame pregnant goats during their first 24 days in the laboratory

	Days in the laboratory					
	1	2	4	7	12	24
Glucose (mg/100 ml)						
Untamed	76	78	73	61	63	59
	±17	±4	±5	±3	±3	±3
Tame	67	67	65	63	62	58
	±2	±4	±3	±1	±2	±2
Corticosteroids (ng/ml)						
Untamed	33	23	32	26	17	15
	±8	±6	±6	±6	±3	±1
Tame	19	17	17	15	—	18
	±3	±2	±2	±3		±3

Experiment 2

The eight untamed sheep in this experiment were extremely excitable at first and only became tame by the fifth or sixth week. Four of them were handled and bled from the first day in the laboratory and had high corticosteroid concentrations (mean 28±7 (4) ng/ml) during the first week. During the following five weeks corticosteroid concentrations were consistently lower (19±2, 19±2, 22±1, 17±2 and 16±1 (4) ng/ml, respectively) and were not significantly different from those of the other four sheep which were not bled and handled until they has been in the laboratory for two weeks. Therefore, the higher plasma corticosteroid concentrations in sheep during their first week in the laboratory were probably due more to the change of environment than to blood sampling and handling procedures. Plasma glucose concentrations (range, 54–69 mg/100 ml) showed no consistent trend in either group and all animals ate the whole of their daily ration of pelleted feed within a week of entering the laboratory.

Experiment 3

The four ewes in this experiment were those used in experiment 2 which were bled and handled from the day they entered the laboratory (day 1). The mean (±SE) of the corticosteroid concentrations of the first samples taken in each of the six venipuncture experiments have been given above (experiment 2). Repeated venipuncture was associated with a transient rise in plasma corticosteroid concentration, the magnitude and duration of which decreased each week until little change was observed. A maximum increment of 19±4 ng/ml occurred on day 1 when the rise above the initial value remained significant for 45 min. Thereafter, increases in corticosteroid concentration of 10±1 ng/ml over 45 min (day 8), 8±2 ng/ml over 45 min (day 15), 9±4 ng/ml over 30 min (day 22) and 8±3 ng/ml over 15 min (day 29) were observed. There was no change on day 36. These animals became tame between days 29 and 36. Although similar changes in corticosteroid concentrations during repeated blood sampling have been reported (Bassett and Hinks 1969; McNatty and Young 1973) the period of taming required for the transient rise to disappear in individual animals was not assessed.

Experiment 4

Heart rate measurements began on the fifth day (day 5) the four ewes were in the laboratory and before this they were not handled. Mean basal heart rate (HR_1) was 69±7 beats/min on day 5 and 60±4 on day 6. Thereafter until day 50 HR_1 remained between 59±3 and 65±2. During the first few days heart rate increased when the pen was entered. This rise ($HR_2 - HR_1$) was 23±9 beats/min on day 5 but by day 10 it had decreased to 5±3 and after two to three weeks little or no change was observed. On days 5 and 6 $HR_2 - HR_1$ was 15±7 and 8±2 beats/min, respectively, but from day 7 it was zero. These four partially tamed ewes showed tame behaviour after two to three weeks of handling, when $HR_2 - HR_1$ decreased to zero.

On day 50 the animals were moved to similar pens in another laboratory. HR_1 increased from 60±1 on day 50 to 69±3 beats/min on day 51, but $HR_2 - HR_1$ and $HR_3 - HR_1$ remained zero. During the following nine days HR_1 decreased to between 62±1 and 65±2 beats/min, rates which were maintained between days 60 and 72.

Discussion

This work demonstrates that without being handled animals will usually adjust to a new laboratory environment within two weeks, unless they react adversely to a marked change in diet (Warner 1962), and will then be usable for experiments involving daily blood sampling. On the other hand, if animals are to remain unstressed by more prolonged experimental procedures three to six weeks of daily handling will be required. There was a close correlation between the disappearance of transient rises in plasma corticosteroid concentrations and heart rates and the appearance of tame behaviour, and thus the criteria of tameness used here seem to be satisfactory indices of the readiness of animals for experiment.

The small rise in basal heart rate which occurred when tame animals were moved to an almost identical laboratory, shows that animals which have apparently adapted fully to experimental rooms and procedures still remain sensitive to small changes in their conditions. This suggests that if animals are to be moved from holding pens to a laboratory for regular physiological monitoring (Meschia *et al* 1965; Strott *et al* 1974) this procedure should be included in any adaptation schedule.

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Solanum malacoxylon poisoning in pigs

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***Solanum malacoxylon* was given orally to four pigs. The animals were examined clinically and subjected to post mortem examination. Macroscopic lesions were not seen with the exception of a small calcified plaque in the endocardium of one animal. Microscopic examinations revealed slight calcification of elastic fibres in the soft tissues. The pathological changes in the bones were extensive and are described in detail. The pigs showed minimal lesions at dose levels which cause considerable systemic calcification in cattle and sheep.**

THE INGESTION OF *Solanum malacoxylon* by cattle is responsible for the disease known as *enteque seco* in Argentina (Carrillo and Worker 1967; Instituto Nacional de Tecnologia Agropecuaria 1967) and *espichamento* in Brazil (Döbereiner *et al* 1971). The disease occurs on lowland pastures and is accompanied by progressive weight loss, locomotory disturbances and progressive systemic calcification, principally of blood vessels, kidneys and lungs. When plant material is administered to cattle absorption of dietary calcium is greater than in controls (Sansom *et al* 1971) and serum calcium and phosphorus concentrations increase.

The natural disease has been described in cattle and sheep (Gaggino 1969; Worker and Carrillo 1967) and it has been produced experimentally in several laboratory animal species, including rabbits (Rossi *et al* 1969), guinea pigs (Camberos *et al* 1970) and rats (Gaggino *et al* 1967) but there are no reports of the toxicity of the plant to pigs. This paper reports the results of feeding *Solanum malacoxylon* to four pigs and compares their susceptibility with that of other species.

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Materials and methods

Solanum malacoxylon leaves were collected from the Pantanal region of Poconé, Mato Grosso, Brazil and stored as a dry powder. Doses of 0·2 or 1·0 g of the dry powder per kg body weight per week were divided into seven equal daily doses, mixed with dry meal and fed to four male Large White pigs. Pig A, 140 days of age at the start of the experiment, received 1·0 g/kg/week for 126 days and a total of 1350 g was consumed. Pig B, 140 days of age at the start of the experiment received 0·2g/kg/week for 128 days, and then after a lapse of 69 days 1·0 g/kg/week for 33 days; a total of 810 g was consumed. Pig C, 45 days of age at the start of the experiment, received 0·2 g/kg/week for 100 days and a total of 845 g was consumed. Pig D, a boar of 710 days of age, received 1·0 g/kg/week for 185 days and a total of 6760 g was consumed.

Four similar pigs were used as controls. All eight animals were examined daily and blood samples taken

SHORT COMMUNICATIONS

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The composition of ruminal and abomasal fluid from catheterised fetal sheep during the last 50 days of pregnancy

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A catheterisation technique for obtaining fluid samples from the rumen and abomasum of fetal sheep between 90 and 140 days gestation is described. The osmolalities and sodium concentrations were higher and the potassium concentrations lower in the ruminal and abomasal fluids than in amniotic fluid, and the mean pH of the amniotic, ruminal and abomasal fluids was 7·0.

THE SHEEP FETUS swallows 250–480 ml of fluid per day (Bradley and Mistretta 1973) but produces little meconium. Its gastrointestinal tract must therefore absorb both water and solutes. In the anaesthetised animal the abomasum and intestines absorb fluid (Wright and Nixon 1961). Whether the rumen modifies the fluid entering it from the amniotic sac, however, is unknown. We have examined this possibility by comparing the osmolalities and the concentrations of sodium and potassium of the amniotic, ruminal and abomasal fluids obtained by means of a catheterisation technique, and have also measured pH to assess whether acid secretion by the abomasum starts before birth.

Materials and methods

Five Scottish Blackface ewes (50–60 kg) were used, three carrying single and two carrying twin fetuses of known age. Under general anaesthesia an incision was made in the uterine wall and fetal membranes, and the hindlegs and abdomen of a fetus were drawn through to expose the lateral area immediately posterior to the last rib on the left side. A 1·5–2·0 cm incision was made through the abdominal wall parallel to and 0·5 cm from this last rib, extending dorsally to a point 1·5–2·0 cm from the spinal column. The reticulo-rumen, which was identified by its rounded appearance, protruded through the incision when gentle pressure was applied to the abdomen close to the incision. The abomasum, which was long and tapered at the pyloric end, was usually located posterior to the ventral end of the incision. After the rumen had been catheterised the abomasum was drawn through the incision and catheterised 2–3 cm from the pylorus. The catheters inserted into the rumen and abomasum had an internal diameter 0·86 mm or 1·00 mm. Other procedures used before, during and after the operation and the sampling procedures were as described by Mellor *et al* (1972).

Osmolality was measured by freezing point depression* and pH by using a small total immersion electrode†.

* Precision Osmometer. Precision Systems, USA

† Radiometer, Copenhagen; combined electrode attached to pH meter model 27

Sodium and potassium concentrations were determined directly in maternal plasma and amniotic fluid and in diluted samples of ruminal and abomasal fluid as described by Mellor and Slater (1973). The viscosity of the ruminal and abomasal fluid samples prevented volumetric analysis, so a known weight of sample was diluted with distilled water and electrolyte concentrations were expressed on a weight basis. There were no differences in the concentrations of sodium or potassium determined on a volume or weight basis in 10 maternal plasma and 10 amniotic fluid samples; all concentrations are therefore reported in mEq/kg fluid.

For simplicity we have referred to fluid from the reticulo-rumen as ruminal fluid. At operation the reticulo-rumen and the abomasum each contained 10 to 15 ml of fluid.

Results and discussion

Five fetuses were catheterised at 87 to 96 days gestation and were born between 145 and 147 days. The course of parturition, birth weights of the lambs (3·1 to 4·9 kg) and their growth rates to eight weeks of age (1·0 to 2·7 kg/wk) were normal. The ruminal and abomasal fluids were viscous and in three fetuses the 0·86 mm catheters blocked after 25 to 30 days while in two fetuses the 1·00 mm catheters remained patent for 40 to 50 days.

Osmolalities and sodium concentrations were generally higher and potassium concentrations lower in the ruminal and abomasal fluids than in amniotic fluid (Table 1); the potassium difference was less marked before 120 days of gestation than subsequently. As fetal plasma samples were not taken, values for the osmolality and the concentrations of sodium and potassium of fetal plasma were derived from the maternal values (Table 1) using the maternal/fetal ratios reported by Mellor and Slater (1971). A comparison using these derived values suggests that amniotic fluid was hypotonic to fetal plasma which was itself hypotonic to both ruminal fluid and abomasal fluid, and that fetal plasma, ruminal fluid and abomasal fluid concentrations of sodium and potassium would not have been significantly different.

These data demonstrate that the composition of amniotic fluid was modified during its passage between the amniotic sac and the rumen. Fetal lung fluid may have been involved, because 100 to 200 ml are secreted each day (Adamson *et al* 1973), and even if 'bouts' of swallowing (Bradley and Mistretta 1973) do not coincide with opening of the laryngeal sphincter and the associated flow of lung fluid into the pharynx (Adams *et al* 1967), lung fluid that subsequently accumulated in the pharyngeal and buccal cavities would mix with amniotic fluid

TABLE 1: Mean (±SD) values for osmolality (m-osmole/kg water) and sodium and potassium concentrations (mEq/kg fluid) of maternal plasma (MP) and of amniotic fluid (AMF), ruminal fluid (RU) and abomasal fluid (ABO) in five fetuses between 91 and 140 days gestation, except for RU which was obtained between 91 and 130 days. The significance of differences between AMF and RU, RU and ABO, and MP and ABO are shown

	AMF	RU	ABO	MP
Osm	264±22 (134)	326±52 (103)***	337±55 (93)^{ns}	279±10 (142)***
Na	118±2 (105)	136±19 (72)***	140±18 (69)^{ns}	145±2 (123)**
K	8·1±1·6 (105)	6·3±1·8 (72)*	6·0±1·6 (69)^{ns}	5·2±0·1 (123)^{ns}

MP osmolality was significantly higher ($P < 0·05$) than that of AMF

^{ns} not significant, * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$

The number of observations are given in brackets

during the next period of swallowing. Such a process could account for the different electrolyte compositions of ruminal and amniotic fluid but cannot explain the hypertonicity of ruminal fluid because lung fluid and plasma have similar sodium and potassium concentrations and are isotonic (Adamson *et al* 1969). Therefore, unless the fetal sheep secretes saliva that is markedly hypertonic to plasma (and there is apparently no information on this point although adult saliva is isotonic (Kay 1960)), the high osmolality of ruminal fluid must result from net secretion of solutes into the ruminal lumen or net absorption of water from it, or both. Further evidence for the presence of such ruminal processes is the greater day-to-day fluctuations in the osmolality of ruminal fluid compared to amniotic fluid (indicated in Table 1 by the different standard deviations), which suggests different lengths of exposure of the fluid to secretory or absorptive processes in the rumen. Since swallowing occurs intermittently (Bradley and Mistretta 1973) the interval between sampling and the last period of swallowing in the present fetuses would have varied. The compositions of the ruminal and abomasal fluids were not significantly different (Table 1), so any further alterations to fluid composition by the omasum and abomasum were apparently less marked.

From 87 to 143 days gestational age the pH of the amniotic, ruminal and abomasal fluids remained relatively constant with means (± SD) of 6·96 ± 0·17 (144), 6·95 ± 0·15 (77) and 6·98 ± 0·15 (49), respectively. Therefore, hydrogen ion secretion by the fetal abomasum did not exceed the buffering capacity of abomasal fluid during this period.

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PROOF

Changes in fetal fluid composition during the last 60 days of gestation in goats

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Although the fetal fluids of sheep have been examined in detail in conscious catheterized animals after about 60 days of pregnancy (Mellor & Slater, 1971, 1972, 1973a; Mellor & Matheson, 1977) there is no comparable information for goats. We have therefore examined the composition of the fetal fluids from conscious goats for comparison with the sheep data.

Twenty-five pregnant British Toggenburg × mixed breed goats (40–80 kg) at known stages of pregnancy were used; 15 carried twins, 9 had single fetuses and 1 triplets. The animals were penned individually and received 0·8–1·0 kg pelleted feed (Ruminant A: Seafield Mill, Midlothian) and 0·3–0·5 kg hay per day. They were handled daily, including sampling from a jugular vein, for 4 weeks and were fasted for 48 h before operation. Between 81 and 111 days of gestation catheters were inserted into the allantoic and/or amniotic sacs of 36 fetuses (Mellor, 1970a) and into the bladders of 3 of them (Mellor, Williams & Matheson, 1972). Fetal fluids, fetal urine and maternal plasma were sampled daily (Mellor *et al.*, 1972) before feeding at 09.30–10.30 h, until term or abortion. Eleven goats (Group A) received no progesterone, 10 (Group B) were given 20 mg progesterone (Organon Laboratories Ltd., Surrey) i.m. in oil once daily from the day before until 3–5 days after operation, the dose then being decreased progressively to zero during the next 2–4 days, and 4 goats (Group C) were given 10–15 mg progesterone/day from the day of operation until term. All analytical procedures were as described by Mellor & Slater (1973b), apart from those for fructose (Roe, 1934) and calcium and magnesium (Mellor & Matheson, 1977). Progesterone concentrations were measured using a modified competitive protein-binding assay based on those described by Thorburn, Bassett & Smith (1969) and Challis, Heap & Illingworth (1971). The extraction effciency was $89 \pm 1{\cdot}3\,\%$ (S.E.M.) ($n = 14$), the values for distilled water blanks were always <0·2 ng/ml, and the interassay coefficient of variation was 20%.

Abortion occurred 2–3 days after surgery in 4 of 11 goats in Group A, after 8–13 days in 6 of 10 goats in Group B and in none of the 4 goats in Group C. During the last 2 days before abortion the mean (±S.D.) plasma progesterone concentration decreased from $7{\cdot}6 \pm 4{\cdot}2$ to $0{\cdot}8 \pm 0{\cdot}5$ ng/ml ($N = 10$), and this decrease started within 24 h of surgery in the Group A animals. All aborted fetuses were alive at delivery, and the likely cause of abortion was therefore lutolysis induced by the stress of surgery. Although short-term progesterone treatment appeared to delay abortion in Group B, the frequencies of abortion in Groups A and B were not significantly different (χ^2 test). Of the remaining 15 animals, the pregnancies of 3 were terminated 3–4 weeks after operation because of persistent sampling difficulties, and 12 (including all 4 from Group C) carried their fetuses to full term (148–155 days) and gave birth spontaneously. The 18 kids of which 13 were catheterized, had birthweights of 2·2–3·3 kg and growth rates during the first 5 weeks of 0·9–1·1 kg/week.

Postoperative changes in amniotic fluid and in allantoic fluid were qualitatively similar in each fetus whether abortion occurred or not, and were similar to those reported for sheep (Mellor & Slater, 1971, 1973a; Mellor & Matheson, 1977). In the animals which did not abort the time required for the establishment of stability or of subsequently observed gestational trends varied according to the substance being considered, but was never more than 10 days in any goat. It took up to 12 days for maternal plasma composition to stabilize, and thereafter the concentrations of all substances remained relatively constant (see Table 1).

During the last 70 days of pregnancy changes in the composition of amniotic fluid were qualitatively similar in 6 fetuses carried by 5 goats in Groups A and B (Table 1). Few samples were obtained

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Table 1. Ten-day interval means for the osmolality and the concentration of sodium, potassium, chloride, fructose, urea, calcium and magnesium in amniotic fluid from 6 goat fetuses after post-operative stabilization.

Gestational age (days)	No. of observations	No. of fetuses	Osmolality (mosmol/kg water)	Na (mM)	K (mM)	Cl (mM)	Fructose (mM)	Urea (mM)	Ca (mM)	Mg (mM)
Amniotic fluid										
81–90	2–14	3	—	129	5·1	123	8·4	—	3·08	0·46
91–100	21–53	6	300	129	5·4	124	11·1	2·70	2·93	0·58
101–110	31–45	6	310	120	7·0	121	18·1	2·63	3·05	0·71
111–120	21–49	6	313	111	8·9	117	15·6	4·23	2·80	1·04
121–130	23–35	6	327	107	9·2	113	14·2	5·62	2·36	1·46
131–140	12–22	3	309	97	15·3	112	12·3	5·92	1·85	1·42
141–150	6–9	2	292	91	23·8	103	12·2	9·20	1·83	1·58
Average S.D.			29	6·08	1·37	6·78	4·22	0·90	0·79	0·42
Maternal plasma										
81–150	125–281	5	280	139	3·98	112	N.D.	2·60	2·83	1·13
		S.D.	10	4·7	0·34	6·2		0·65	0·48	0·24

N.D., not detectable.

after 140 days of gestation because the fluid became increasingly gelatinous and blocked most catheters.

Allantoic fluid was sampled for 20–45 days in 7 single fetuses, including the 4 from Group C. Few samples were obtained after 135 days. The osmolality showed a general tendency to increase during the sampling period (mean 330 ± 32 (n = 110) mosmol/kg water). Within each fetus there was a significant negative correlation between the sodium (10–120 mM) and potassium (20–90 mM) concentrations of allantoic fluid (r = −0·50 to −0·98, n = 21–42). Chloride concentrations were 20–45 mM until about 118 days of gestation when they increased continuously to reach a maximum of 87 ± 7 (n = 7) mM between 125 and 135 days. The fluid fructose concentrations showed linear decreases in each fetus, the highest values being 10·0–36.1 mM and the lowest 5·0–18·9 mM. The urea (3·34–6·34 mM) and magnesium (3·42–4·33 mM) concentrations remained relatively constant, but calcium concentrations decreased from 13·0–40·0 mM within 3 days of operation to 2·50–11·3 mM after 20–45 days.

Daily samples of fetal urine were obtained from 3 fetuses until abortion 8, 9 and 13 days after operation. The results for each parameter between the 1st and 7th day after operation were pooled and gave mean ± S.D. (n) values of 347 ± 102 (20) mosmol/kg water, 59 ± 11 mM-sodium (21), 6·9 ± 2·3 mM-potassium (21), 43 ± 12 mM-chloride (21), 14·3 ± 9·4 mM-fructose (21) and 9·67 ± 4·17 mM-urea (21). Although the operation probably affected these values they have been included because they are the only data available.

Discussion

During the last 60 days of pregnancy the changes in the composition of amniotic fluid in the goats in the present study (Table 1) and the sheep examined by Mellor & Slater (1971, 1973a) were almost identical. Because the relative compositions of fetal urine and amniotic fluid in both species were also similar, it may be argued that, in the goat, as in the sheep, fetal urine flows at increasing rates into the amniotic sac after 80–90 days of gestation. Such a flow would account for the high osmolality and the changes in the sodium, potassium and urea concentrations of amniotic fluid, but would need to be combined with decreasing fructose concentrations in fetal urine to account for the decrease in the fluid fructose concentrations after 110 days of gestation.

In the sheep, it has been suggested that chloride ions are actively transported from fetal plasma into amniotic fluid until about 130–135 days of gestation, after which chloride pumping apparently decreases (Mellor, 1970b; Mellor & Slater, 1971). Because the electrochemical gradients between fetal plasma and amniotic fluid (Mellor, 1970b) and the gestational trends in the fluid chloride concentrations (Mellor & Slater, 1971) are similar in goats and sheep, a similar pattern of chloride pumping

presumably occurs in the goat. As in the sheep (Mellor & Matheson, 1977), the calcium and magnesium concentrations of amniotic fluid would be determined largely by a balance between their diffusion into the amniotic sac down the electrochemical gradient generated by chloride pumping and effects of the entry of fetal urine into the amniotic sac.

Changes in the composition of goat allantoic fluid (present study) were also similar to those observed in sheep (Mellor & Slater, 1971, 1973a; Mellor & Matheson, 1977). However, although there were negative correlations between the fluid sodium and potassium concentrations in both species, the changes in the goats with gestation were markedly different from those in sheep (Mellor & Slater, 1971, 1972). Higher chloride concentrations (60–90 mM) were found in allantoic fluid from conscious goats than were normally present in fluid from conscious sheep (usually <50 mM, Mellor & Slater, 1971) or anaesthetized goats (Mellor, 1970b), and the rise in chloride concentration to these levels coincided with an increase in the sodium and a decrease in the potassium concentrations of the fluid. Mellor (1970b) has suggested that relatively low chloride concentrations in allantoic fluid are maintained passively by the electrochemical gradients between maternal and fetal plasma and allantoic fluid, and that these gradients are generated by sodium and possibly potassium pumps in the chorioallantoic membrane. The usual electrolyte changes after 118 days in fluid from the present animals could therefore have resulted from an increase in the permeability of the chorioallantois to these ions, or to a decrease in sodium pumping activity, or both. Since these electrolyte changes occurred in the fluid from all 7 goats, the daily progesterone injections in the 4 Group-C animals are not likely to have been involved. Indeed, the general compositions of allantoic fluid from the animals in the three treatment groups were indistinguishable.

We conclude that the factors which regulate or alter the compositions of the fetal fluids, particularly the amniotic fluid, of sheep and goats during the last half of pregnancy are probably similar.

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